

**MOLECULAR MAPPING OF
GENETIC LOCI ASSOCIATED WITH
TOLERANCE TO LOW SOIL
PHOSPHORUS IN RICE (*Oryza sativa*
L.)**

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M. Sc. (Ag.)

**DOCTOR OF PHILOSOPHY IN AGRICULTURE
(MOLECULAR BIOLOGY AND BIOTECHNOLOGY)**



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BY

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M. Sc. (Ag.)

**PROFESSOR JAYASHANKAR TELANGANA STATE
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CHAIRPERSON: Dr. R. M. SUNDARAM



INSTITUTE OF BIOTECHNOLOGY

COLLEGE OF AGRICULTURE

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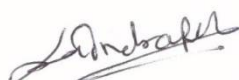
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AGRICULTURAL UNIVERSITY**

2020

DECLARATION

I, **KALE RAVINDRA RAMRAO**, hereby declare that the thesis entitled
“**MOLECULAR MAPPING OF GENETIC LOCI ASSOCIATED WITH
TOLERANCE TO LOW SOIL PHOSPHORUS IN RICE (*Oryza sativa* L.)**”
submitted to **Professor Jayashankar Telangana State Agricultural University** for the
Degree of **Doctor of Philosophy in Agriculture** is a result of original research work
done by me. I also declare that no material contained in the thesis has been published
earlier in any manner.

Place: *Hyderabad*
Date: *11/02/2020*


(**KALE RAVINDRA RAMRAO**)
I. D. No.: RAD/14-43

CERTIFICATE

Mr. **KALE RAVINDRA RAMRAO** has satisfactorily prosecuted the course of research and that thesis entitled “**MOLECULAR MAPPING OF GENETIC LOCI ASSOCIATED WITH TOLERANCE TO LOW SOIL PHOSPHORUS IN RICE (*Oryza sativa* L.)**” submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that neither the thesis nor its part thereof has been previously submitted by him for a degree of any University.

Place: Hyderabad

Date: 11/02/2020.



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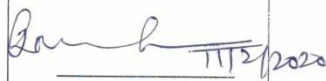
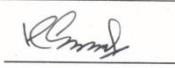
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CERTIFICATE

This is to certify that the thesis entitled "**MOLECULAR MAPPING OF GENETIC LOCI ASSOCIATED WITH TOLERANCE TO LOW SOIL PHOSPHORUS IN RICE (*Oryza sativa* L.)**" submitted in partial fulfillment of the requirements for the degree of 'Doctor of philosophy in Agriculture' of the Professor Jayashankar Telangana State Agricultural University, Hyderabad is a record of the bonafide original research work carried out by **Mr. KALE RAVINDRA RAMRAO** under our guidance and supervision.

No part of the thesis has been submitted by the student for any other degree or diploma. The published part and all assistance received during the course of the investigations have been duly acknowledged by the author of the thesis.

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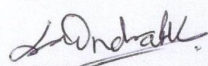
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LIST OF CONTENTS

Chapter No.	Title	Page No.
I	INTRODUCTION	
II	REVIEW OF LITERATURE	
III	MATERIAL AND METHODS	
IV	RESULTS AND DISCUSSION	
V	SUMMARY AND CONCLUSIONS	
	LITERATURE CITED	
	APPENDICES	

LIST OF TABLES

Table No.	Title	Page No.
2.1	List of QTLs associated and molecular markers identified for low soil P condition from diverse mapping populations in rice	
3.1	List of germplasm lines used for checking the allelic status among the germplasm lines with respect to <i>Pup1</i>	
3.2	Status of Soil Nutrients and pH in low soil P plot at ICAR-IIRR, Hyderabad during <i>Kharif</i> - 2017	
3.3	Details of primers used for checking the allelic status among the germplasm lines with respect to <i>Pup1</i>	
4.1	Performance of rice germplasm lines in low soil P condition at ICAR-IIRR, Hyderabad during <i>Kharif</i> -2017	
4.2	Analysis of variance for growth and yield parameters of the germplasm lines grown under Low soil P plot at ICAR-IIRR, Hyderabad during <i>Kharif</i> -2017	
4.3	Performance of rice germplasm lines in normal soil P condition at ICAR-IIRR, during <i>Kharif</i> , 2017	
4.4	PCV, GCV, Heritability and Genetic advance for different traits of germplasm lines grown under low soil P plot at ICAR-IIRR, during <i>Kharif</i> -2017	
4.5	Stress indices calculated for germplasm lines based on single plant yield under normal and low soil P conditions	
4.6	Clustering of rice germplasm lines into different groups based on <i>Pup1</i> specific markers analysis	
4.7	Chromosome wise list of SSR markers used and polymorphic SSR markers identified for QTL mapping	
4.8	Descriptive statistic data of yield and yield related traits obtained under low soil P condition	
4.9	Descriptive statistic of yield and yield related traits obtained under normal soil P condition	

Table No.	Title	Page No.
4.10	Values of the significant and positive correlation for yield and yield related traits in Improved Samba Mahsuri X Wazuhophek RIL population under low soil P condition	
4.11	QTLs detected for low soil P tolerance in RIL population of Improved Samba Mahsuri X Wazuhophek	
4.12	Comparative table of QTL analysis for low soil P tolerance with RILs population using ICIM qtl mapping and WInQTL cartographer mapping software	
4.13a	List of annotated genes present within QTL intervals on chromosome 1, related to phosphorus utilization and uptake	
4.13b	List of annotated genes present within QTL intervals on chromosome 7, (23 to 27mb genomic region) related to phosphorus utilization and uptake	
4.13c	List of annotated genes present within QTL intervals on chromosome 7 (4 to 16.5 mb gnomc region) related to phosphorus utilization and uptake	
4.13d	List of annotated genes present within QTL intervals on chromosome 8 related to phosphorus utilization and uptake	
4.14	Epistatic interaction observed for various traits studied for low soil P tolerance	

LIST OF ILLUSTRATIONS

Fig. No.	Title	Page No.
2.1	Phosphorus availability across pH ranges	
4.1a	Evaluation of germplasm lines with parental lines during Kh17 at low soil P screening plot of ICAR-IIRR	
4.1b	Screening of rice germplasm lines under low P soils and performance of lines growing under low soil P plot at ICAR-IIRR during Kh17	
4.2	Clustering pattern of germplasm lines based on stress indices under low P (< 2 kg P ha ⁻¹) and normal P (60 kg P ha ⁻¹) soils	
4.3	Genotyping of rice germplasm lines using <i>Pup1</i> locus specific markers	
4.4	Schematic representation of crossing program for development of RIL mapping population (Improved Samba Mahsuri X Wazuhophek)	
4.5	Parental Polymorphism analysis between Wazuhophek (W) and Improved Samba Mahsuri (I)	
4.6	Screening of mapping population in low P soil plot at ICAR IIRR, Hyderabad	
4.7	Phenotypic distribution of RILs for various low soil P related traits under low soil P field condition	
4.8	Segregation pattern of polymorphic SSR markers showing co-segregation with tolerance in the RIL mapping population	
4.9a	Linkage map and the position of identified QTL for low soil P tolerance (Chromosome 1 to chromosome 6)	
4.9b	Linkage map and the position of identified QTL for low soil P tolerance (Chromosome 7 to chromosome 12)	
4.10	Epistatic interaction observed for various traits studied for low soil P tolerance	

LIST OF SYMBOLS AND ABBREVIATIONS

@:	At the rate
~:Nearly	
<:	Less than
AFLP:	Amplified Fragment Length Polymorphism
ANOVA	: Analysis of variance
bp	: Base pairs
CIM	: Composite Interval Mapping
cm	: Centimeter
cM	: Centimorgan
CTAB	: Cetyl amino borate
CV	: Coefficient of variation
DF	: Days to Fifty per cent flowering
DNA	: DeoxyRibo Nucleic acid
dNTP	: Deoxyribose Nucleoside Tri-Phosphate
Df	: Degrees of freedom
EDTA	: Ethidiumdiamino tri acetate
Et Br	: Ethidium Bromide
<i>et al.</i>	: and coworkers
Ex	: Exon
F ₁	: First filial generation of a cross
F _{6:7}	: F ₂ derived F ₃ population
Fig	: Figure
g	: Grams
ha	: Hectare
H	: Heterozygous
ICAR	: Indian Council of Agricultural Research
<i>i.e.</i>	: That is
IIRR	: Indian Institute of Rice Research
IM	: Interval Mapping
InDels	: <i>insertion-deletion</i> polymorphisms
K	: Potassium
kg	: Kilogram
kg ha ⁻¹	: Kilogram per hectare
LOD	: Logarithm of Odds Ratio

MAS	:	Marker-assisted selection
MABC	:	Markers assisted backcrossing
Mb	:	Magabases
mM	:	milli molar
mg ml ⁻¹	:	milligram per milli litre
mha	:	million hectare
min	:	minutes
ml	:	milli litre
mm	:	milli meter
N	:	Nitrogen
NaCl	:	Sodium chloride
NaOH	:	Sodium Hydroxide
Ng	:	Nano gram
no.	:	Number
P	:	Phosphorous
PCR	:	Polymerase Chain Reaction
PIC	:	Polymorphism Information Content
pH	:	Power of Hyderogen
PL	:	PanicleLength
PVE	:	Phenotypic Variance Explained
No. of	:	Number of
QTL	:	Quantitative Trait Loci
RAPD	:	Randomly amplified polymorphic DNA
RFLP	:	Restriction fragment length polymorphism
RILs	:	Recombinant Inbred Lines
RM	:	Rice Microsatellite
RPM	:	Revolution per minute
RNA	:	Ribo Nucleic Acid
sec	:	Seconds
SMA	:	Single Marker Analysis
SNP	:	Single Nucleotide Polymorphism
SR	:	Soft Rice
SSD	:	Single seed descent
SSRs	:	Simple Sequence Repeats
STS	:	Sequence tagged sites
TE	:	Tris EDTA
<i>Taq</i>	:	<i>Thermusa quaticus</i>
TAE	:	Tris Acetate EDTA
t /ha	:	Tones per hectare

V	:	Volt
viz.,	:	Namely
%	:	per cent
⁰ C	:	Degree centigrade
$\bar{X}$	:	Grand mean
⁻¹	:	Per
μl	:	micro liter
-	:	Minus

ABSTRACT

Author : KALE RAVINDRA RAMRAO

Title of Thesis : MOLECULAR MAPPING OF GENETIC LOCI ASSOCIATED WITH TOLERANCE TO LOW SOIL PHOSPHORUS IN RICE (*Oryza sativa* L.)

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ABSTRACT

Rice is one of the most important cereal crops and occupies second prominent position in global agriculture. A significant proportion of rice growing soils across the world and in India is either phosphorus (P)-deficient or has a high P-fixing capacity resulting in reduced productivity levels. The basic mechanism plants follow for P utilization are P acquisition efficiency and internal P-use efficiency. Presence of genetic variability for P uptake has been reported in rice and a major QTL called *Pup1* conferring tolerance to low soil P has been identified earlier. The present study was taken up in order to characterize available germplasm lines for low P tolerance and QTL mapping for low soil P tolerance in recombinant inbred line (RIL) mapping population developed by crossing, Wazuhophek (which has significant tolerance to low soil P condition and completely devoid of *Pup1* genomic region) and Improved Samba Mahsuri (ISM; Which is highly sensitive to low soil P).

In the first objective, i.e. a set of 56 germplasm lines of rice were analyzed for their genotype with respect to markers spanning the *Pup1* locus along with phenotypic characterization for low soil P tolerance. Significant genetic variability was observed with respect to plant height, number of productive tillers, root volume, dry root weight, dry shoot weight and grain yield under low P condition. Cluster analysis based on the stress indices and low soil P tolerance ability, grouped the genotypes into three groups, viz., highly tolerant, tolerant and sensitive. Genotyping of the rice lines with a set of *Pup1* specific markers (viz., K46-1, K46-2, K52 and 46CG-1), revealed the presence of partial or complete *Pup1* genomic region in most of the tolerant genotypes. A total of 15 genotypes showed tolerance to low soil P based on yield reduction in comparison to the tolerant and sensitive checks. Interestingly, the tolerant genotype line, IC216831 was observed to be completely devoid of *Pup1*, and hence it can be explored for identification and characterization of new loci underlying low soil P tolerance.

The second objective focused on SSR marker-based molecular mapping of genomic regions associated with low soil P tolerance in the non-*Pup1* type, low P tolerant rice line from North-eastern part of India, Wazuhophek. Quantitative trait loci (QTLs) related to low soil P tolerance were identified in recombinant inbred lines (RILs), developed from cross Improved Samba Mahsuri/Wazuhophek. A total of 16 QTLs related to yield and other physio- morphological traits were identified under low soil P condition. Out of them, 10 QTLs were found on chromosome 8, spanning a 4.49 Mb region in the genomic interval of RM22554-RM8005. Two QTLs were identified on chromosome 7, spanning a 2.71 Mb (RM5847-RM22031) region and 11.31 Mb (RM21521-RM21103) region for root volume and P content of grains, respectively. Four QTLs were identified on chromosome 1, spanning a 9.2 Mb region in between the markers WR1.7-RM8105.

In silico analysis of identified genomic regions spanning the identified QTLs was carried out. A total of 538 genes were predicted in the QTL region on chromosome 8, of which eight genes related to phosphorus uptake or utilization, including genes related to auxin signalling, phosphatase activity and zinc finger transcription factor appear to be of interest.

A total of 1339 genes were predicted in the QTL region on chromosome 1, of which 14 genes related to phosphorus uptake or utilization, including auxin responsive genes and genes involved in maintenance of Pi homeostasis are of interest. A total of 712 genes were predicted in the QTL region controlling root volume, located on chromosome 7. Among them, 12 genes related to phosphorus uptake or utilization, including auxin responsive genes and transcription factors involved in regulation of stress related genes appear to be of interest. Similarly, *in silico* analysis of another QTL for P content in seed located on chromosome 7, predicated 570 genes. Among them, 16 were found to be possibly related to phosphorus uptake or utilization. All the QTLs detected in the present study, appear to be novel and did not overlap with the already reported genes/QTL. The novel low P tolerant rice line, IC216831, which is devoid of *Pup1* can be subjected for genetic and molecular marker analysis to identify novel low P tolerance genes/QTLs. Further, the novel QTLs associated with low soil P tolerance identified from Wazuhophek, after their fine-mapping and validation, can be transferred into elite rice varieties and parental lines, which are sensitive to low soil P levels through marker-assisted breeding.

REVIEW OF LITERATURE

Chapter II

REVIEW OF LITERATURE

2.1. Rice production and limiting factors

Rice (*Oryza sativa* L.), a cereal with smaller genome of 389Mb, enriched genetic map, available genome sequence data, large collection of germplasm has become a model crop species. China and India, which account for more than one-third of global population supply over half of the world's rice. India stands first in terms of area under cultivation and second in production after China. Rice is one of the principal food crops for half of the world population, for about 3.5 billion in the world. Rice is grown in 159.81 million hectares (~12% of the world's cultivated land), with an annual production of 740.96 million tonnes with a productivity of 4.63 tones ha⁻¹ (Food and Agriculture Organization, 2016). China was the world's leading paddy rice producer, followed by India. India ranks first in area (43.39 million hectares), second in production in the world (104.32 million tons) with an average productivity of 2.40 tones ha⁻¹ (Agricultural Statistics at a Glance-2016; http://eands.dacnet.nic.in/latest_2006.htm). Even though the present trend of rice production is in line with the country's requirement, it has been estimated that India and the world needs to produce at least 25% more rice by the year 2030 to meet the estimated demand of an increasing population (Wani and Sah, 2014). Global rice consumption is expected to increase from 450 million tons in 2011 to about 490 million tons in 2020 and to about 650 million tons by 2050 (Rejesus *et al.*, 2012).

As rice is one of the most economically valuable crops in India compared to other crops and provides the principal supply of calories and protein for poor people, Indian rice breeders have been attempting development of rice cultivars with durable resistance to meet the food demand of growing population in an alarmed rate. The predicted water scarcity, arable land, the constant battle against new emerging pathogens and pests and possible adverse effects from climate change and various abiotic stresses are present great challenges for rice breeders and agricultural scientists (Khush, 2005). Various factors including biotic and abiotic stresses, low returns in developing countries and increasing cost of fertilizer as well as public concern for the

protection of environmental resource cause reduction in rice cultivation and production (Van Nguyen *et al.*, 2006; Hasan *et al.*, 2015).

Abiotic stresses such as drought, water-logging, cold, heat, soil salinity and mineral deficiency always negatively impacts growth, development and yield of crop plants. Phosphorus (P) is the second most important inorganic plant nutrient after nitrogen, which is the least available nutrient because of its tendency for tight binding in the soil. P is one of the major yield limiting nutrients for rice and its deficiency leads to greater yield loss (Fageria and Baligar, 1997). Low soil phosphorus (P) levels, interrupts rice crop growth and yield worldwide (Zhang *et al.*, 2014). This deficiency can be corrected through fertilizer application, but farmers in low input farming ecosystems are resource-poor or simply do not have access to P fertilizers due to typical uplands and rain fed lowlands (Kirk, 2005). P deficiency in rice will lead to stunted growth with greatly reduced tillers, narrow leaves and spindly stems with reduction in number of leaves, panicles and grains per panicle. P deficiency also delays flowering and maturity by one week or more, when the deficiency is severe plants may not flower at all and when flowered, large number of empty grains will form with poor grain quality (Dobermann and Fairhurst, 2000). Among the bacterial diseases, bacterial blight caused by *Xanthomonas oryzae* pv. *Oryzae* (Xoo) (Swings *et al.*, 1990) is one of the most devastating diseases of rice world-wide. Among the fungal diseases of rice, rice blast caused by the fungus *Magnaporthe oryzae* (anamorph *Pyricularia oryzae*), is a destructive disease, which leads to significant yield losses up to 70-80% during an epidemic (Khush and Jena, 2009). Brown planthopper (BPH), *Nilaparvata lugens* (Stål) most important pest emerged during 1970s and is a major threat to the rice production in India and Asia (Bentur and Viraktamath, 2008).

2.2 Status of Soil Phosphorous and Phosphorus fertilizer consumption in Rice

Rice (*Oryza sativa* L.) yields are often limited by the low availability of phosphorus (P) in a variety of soils typically found in the tropics (Sanchez and Salinas, 1981). Low levels of plant-available phosphorus (P) in soils are a major constraint for rice (*Oryza sativa* L.) production throughout the world. While this is more pronounced in upland rice, P deficiency has been identified as a main factor in preventing the realization of high yield potentials of modern varieties in lowland rice production as well.

Increased P fertilizer use and livestock production has fundamentally altered the global P cycle. Phosphorus deficiency is widespread in the Indian as well as world soils and estimates forecast for no P-reserves by 2050; as such India does not have adequate reserves of rock phosphate posing threat to sustainable crop production (Dey *et al.*, 2017). Many soils are inherently poor in available phosphorus content (Barber, 1995) although the total amount of P in soil may still be high (Vance *et al.*, 2003) and is evident from the extremely low soil solution P concentration ($<1\ \mu\text{M}$) in sandy soils, alkaline soils, acidic soils and highly weathered soils of tropics and subtropics. Many of the agricultural soils in the developing countries like India in particular are P-deficient (Vlek and Koch, 1992) and have an unfavorable condition for P availability (Soltan *et al.*, 1993). In India 49.3 percent of the soils are low, 48.8 percent are medium and 1.9 percent are high in available P (Hasan, 1996; Tiwari, 2001). P is highly immobile in soil, unlike nitrogen, which readily moves in soil towards the roots via both mass flow and diffusion (Lavakush *et al.*, 2014). Further, a large fraction of total soil P is in organic form in many soils and these forms are not directly available to plants (Smith, 2001; Vance *et al.*, 2003). As a result of adsorption, precipitation and conversion to organic forms, only 10-30% of the applied phosphate mineral fertilizer can be recovered by the crop (Holford, 1997; Syers *et al.*, 2008). Inefficient utilization of phosphate (P) fertilizer by the plant in rice production increase cost and pollution (Wu *et al.*, 2013). Clarkson (1981), reported that the diffusion coefficient for phosphate ion in soil is very low compared to those for other nutrients, so plants do not deplete the total volume of the rooted soil layer but only that part of the soil which is in the immediate vicinity of the roots for P.

Because of the limited solubility fertilizer, phosphorus will become much less available as it reverts back to soil phosphorus compounds. Phosphorus availability is controlled by three factors viz., soil pH, amount of organic matter and proper application of phosphorus fertilizer. Phosphorus deficiency is more common in upland environments, because P predominantly reacts with iron (Fe) and aluminum (Al) in acidic soils, and calcium (Ca) in neutral to alkaline soils to form poorly soluble complexes (Holford, 1997), but is released from some of these compounds after flooding as the redox potential and pH change (Kochian *et al.*, 2004; Jensen, 2010).

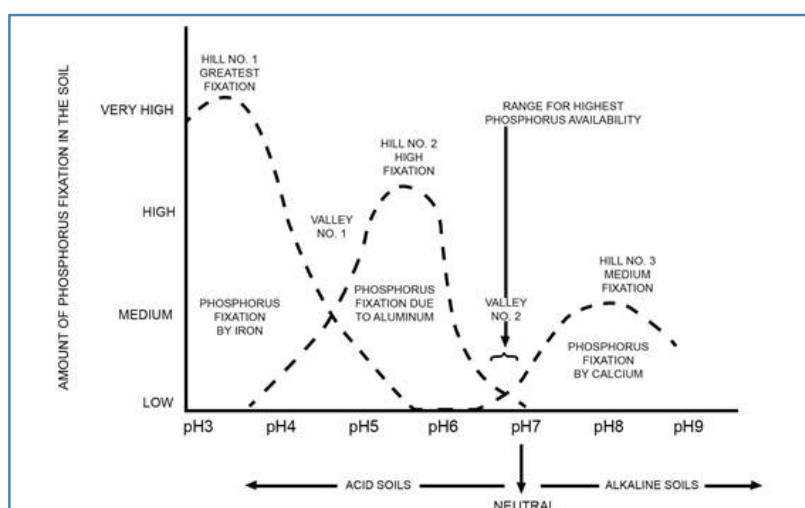
The P nutrition of plants is predominantly controlled by P dynamics in the soil/rhizosphere-plant interaction. The integration of P dynamics from soil to plant via the rhizosphere provides a comprehensive picture of available P behaviour and efficient

acquisition in association with plant adaptive strategies (Shen *et al.*, 2011). Certain microorganisms such as phosphate solubilizing bacteria (*Pseudomonas* sp., *Bacillus* sp., *Micrococcus* sp. etc), fungi (*Aspergillus* sp. *Penicillium* sp. etc.), actinomycetes mostly those associated with the plant rhizosphere are known to convert insoluble inorganic phosphorus into available form (Nautiyal *et al.*, 2000; Choudhary *et al.*, 2014; Mamathashree *et al.*, 2018).

Organic matter maintenance is an important factor in controlling phosphorus availability as mineralization of organic matter provides a steady supply of available phosphorus. Yang *et al.* (2013) carried out study to estimate the global distribution of different forms of P in soils and results showed that initial P concentration in parent material and geochemical processes such as weathering of parent material and the loss of P via leaching are dominant in regulating soil phosphorus cycles in the long term. Estimation of global anthropogenic P, loads to freshwater and the associated grey water footprints (GWFs) showed that more than half of this total load was in Asia, followed by Europe (19%) and Latin America and the Caribbean (13%). The domestic sector contributed 54% to the total, agriculture 38%, and industry 8%. In agriculture, cereals production had the largest contribution to the P load (31%), followed by fruits, vegetables, and oil crops, each contributing 15%. The global total GWF related to anthropogenic P loads with China contributing 30%, India 8%, USA 7%, and Spain and Brazil 6% each calculated (Mekonnen and Hoekstra, 2017).

The consumption of phosphate fertilizer in agriculture has been increased from 41.7 million tonnes in 2013 to 43.8 million tonnes in 2015 and would be 52.9 million tonnes by 2030 (European Commission 2013; Food and Agriculture Organization, 2015; Brears, 2015). India is the second biggest consumer of fertilizer in the world next only to China. India ranks second in the production of nitrogenous fertilizers and third in phosphatic fertilizers. Domestic, Di-ammonium phosphate (DAP) production is growing at a robust rate of 4.41% CAGR from FY 2012-13 to FY 2016-17. DAP production levels have raised by an increase of 14.4% from FY 2015-16 to FY 2016-17, while imports by India have reduced by a fall of 27% of the imports during the FY 2015-16 to FY 2016-17. India is one of the biggest importers of phosphorus based fertilizers with 90% dependency. This precarious scenario could have particularly severe consequences for India. The lack of locally available phosphorus sources and the high cost of importing and transporting phosphorus fertilizers frequently prevent resource poor farmers in developing countries from applying phosphorus to their

deficient fields (Sanchez and Salinas, 1981). Yet the world's main source of phosphorus "phosphate rock" is non-renewable and becoming increasingly scarce and expensive which could be exhausted in the next 50-100 years (Steen, 1998; Gunther, 2005). Peak phosphorus was estimated to occur by 2035, after which demand would outstrip supply (Cordell *et al.*, 2009). Morocco has a near monopoly on Western Sahara's rock phosphate reserves, China is drastically reducing exports to secure domestic supply, US has less than 30 years left of supplies, while Western Europe and India are totally dependent on imports (Jasinski, 2006; Rosmarin, 2004; Cordell *et al.*, 2009). Resolving agronomic P imbalances may be possible with more efficient use of P fertilizers and more effective recycling of manure P. Agricultural P management is central to maximizing agricultural productivity while simultaneously reducing threats to water quality due to P loading from agricultural lands, as well as accounting for uncertainties in future access to inorganic P fertilizers (MacDonald *et al.*, 2011; Dey *et al.*, 2017).



Source: California Fertilizer Association, 1995

Figure. 2.1: Phosphorus availability across pH ranges

2.3 Phosphorus, its importance in plant life cycle and rice

Phosphorus (P) is one of the seventeen essential nutrient elements required for plant growth and development (Raghothama, 1999). Being the second most important macronutrient next to nitrogen, P is essential to plants both as a part of several key plant structure compounds and as a catalysis in the conversion of numerous key biochemical reactions in plants. It is a vital component of nucleic acids (DNA and RNA), ATPs, Lipids (phospholipids) and play a role in transfer of genetic information from generation to generation, transfer and store of energy from sun, cell membrane development and function. Plants use P to modify the activity of various enzymes by

phosphorylation, and also for cell signaling. P is usually concentrated at the most actively growing points of a plant and stored within seeds in anticipation of their germination. Some specific growth factors that have been associated with phosphorus in plants are, stimulation of root development (Bates and Lynch, 1996; Williamson *et al.*, 2001 and Peret *et al.*, 2011), increase of stalk length/plant height, increases nitrogen fixing capacity of legumes (Ahlawat and Saraf, 1981; Lynch *et al.*, 1990), improves flower and seed formation, ensures uniform and earlier crop maturity, increases resistance to plant diseases and supports the development of plant throughout its life cycle.

P deficiency leads to stunted, dark green plants with erect leaves and reduced tillering. Leaves are narrow, short, very erect, and 'dirty' dark green. Stems are thin and spindly, and plant development is retarded. The number of leaves, panicles, and grains per panicle may also be reduced (Gupta *et al.*, 2016). It is mobile within the plant and promotes root development, tillering, early flowering, and ripening (especially where the temperature is low). Phosphorus is remobilized within the plant during later growth stages if sufficient P has been absorbed during early growth. It is critically important to develop crops with tolerance of multiple stresses because, P deficiency is often a secondary effect in soils with high concentrations of iron and aluminum and with low pH, which restrict root growth even if P is available (Ismail *et al.*, 2007). P as an important nutrient which plays important role in the signaling pathways involving Pi status, photo-assimilates, phytohormones and that these interconnections manifest themselves at physiological, biochemical, and molecular levels (Rouached *et al.*, 2013). Cross talk between sugars, microRNAs (miRNA) and P- starvation-induced gene expression will be helpful to understand the fundamental underpinning of plant adaptation to low P stress (Vance, 2010). Therefore, the development and use of genotypes which are P deficiency tolerance, with high P uptake efficiency and P use efficiency are very much crucial to enhance and sustain the rice production.

Dobermann and Fairhurst (2000), reported that P deficiency in rice may leads to stunted growth with greatly reduced tillers, narrow leaves which will be short, erect and dirty dark green in color. Stems become spindly and the number of leaves, panicles and grains per panicle will also reduce. Young leaves appear to be healthy but older leaves turn brown and die. Red and purple colors may develop in leaves if the variety has a tendency to produce anthocyanin. Delays the flowering and maturity by one week or

more, when the deficiency is severe, plants may not flower at all, large number of empty grains will form and grain quality will get affected.

Fageria and Baligar (1997), conducted five green house experiments on an Oxisol to identify nutrient deficiencies in common bean, upland rice, corn, wheat, and soybean. They reported that tops dry matter yield of the entire crop species was affected by fertility treatments but significant effects of treatments were found in the case of common bean, upland rice, and corn. Based on top dry weight under different treatments compared to adequate fertility level (AFL), they observed that the most yield-limiting nutrient was phosphorus (P). They also reported that rice is tolerant to soil acidity to certain extent but highly sensitive to soil P deficiency.

Kavanova *et al.* (2006), used a kinematic method to analyze the effect of phosphorus nutrition status on cell division and elongation parameters in the epidermis of *Lolium perenne*. They observed P deficiency reduced the leaf elongation rate by 39% due to decrease in the cell production rate (219%) and final cell length (220%). According to them former was solely due to a lower average cell division rate (0.028 vs 0.046 cell cell⁻¹h⁻¹) and thus lengthened average cell cycle duration (25 versus 15 h). The number of division cycles of the initial cell progeny (five to six) and, as a result, the number of meristematic cells (32–64) and division zone length were independent of phosphorus status. Accordingly, low-phosphorus cells maintained meristematic activity longer. Phosphorus deficiency did not affect the elongation-only zone length, thus leading to longer cell elongation duration (99 versus 75 h). However, the substantially reduced post-mitotic average relative elongation rate (0.045 versus 0.064 mm mm⁻¹h⁻¹) resulted in shorter mature cells.

Li *et al.* (2009), in their study, they characterized the responses of seedlings to low P availability using a set of 271 introgression lines (ILs), to identify QTLs for root traits, biomass, and plant height under P-deficiency and P-sufficiency conditions. Their study revealed that, plant height, total dry weight, shoot dry weight, and root number were inhibited under P-deficiency, whereas maximum root length (MRL) and root-shoot ratio (RS) were induced by P-deficiency stress. They used relative MRL (RMRL, the ratio of MRL under P-deficiency to MRL under P-sufficiency condition) and relative RS (RRS) to evaluate P-deficiency tolerance at the seedling stage. They identified total of 24 additive QTLs and 29 pairs of epistatic QTLs, but only qRN4 was detected in both conditions. QTLs for adaptive traits (RMRL, RRS, RRV, and RRDW) and qRN4

consistently expressed to increase trait stability may contribute to P-deficiency tolerance.

Chiangmai and Yodmingkhwan. (2011), evaluated the effect of phosphorus application on shoot biomass, root biomass, and shoot/root biomass ratio of common wild rice (*Oryza rufipogon* Griff.) and two tropical cultivated rice (*O. sativa* L.) varieties, under two levels of inorganic phosphorus application (0 mgP/l and 10 mg P/l) both in monoculture (specific variety growing) and mix-culture (genetic competition setting) at early stage of growth (about 45 days after planting). Their results indicated that phosphorus availability at 10 mg P/l clearly affected both rice shoot and root biomass at early growth stage. They found significant interaction effect between phosphorus levels and rice varieties.

Vance (2001), estimated that crop productivity is limited by P deficiency on more than 40% of the world arable lands. Dobermann and Fairhurst (2000), reported wide spread of P deficiency is in all major rice ecosystems, it is the major growth limiting factor in acid and alkaline soils where P-fixation is large. Holford (1997) reported that because of low P solubility and desorption, only a small proportion of phosphate ions exist in the soil solution for plant uptake even under optimum P fertilization making P fertilizer recovery to be lower compared to other nutrient containing fertilizers. This suggests that chemical fertilizer application alone is not a cost effective way of increasing crop production in many P-limiting soils (Tilman *et al.*, 2002).

2.4 Screening for Genetic variation under low soil P

Fageria *et al.* (1988a), screened 25 rice cultivars (*Oryza sativa* L.) at low, medium, and high levels of soil P and observed significant cultivar differences in P use efficiency. They also observed positive correlations among growth parameters such as plant height, tillers, root and shoot weight, and P content of roots and shoots. These results indicates, selection of rice cultivars for satisfactory performance under low P availability can be carried out using shoot and root dry weight as criteria.

Fageria *et al.* (1988b), evaluated responses of 75 genotypes of upland rice at two soil P levels in two field experiments. They found that rice cultivars differed significantly in shoot dry matter production at flowering, grain yield, and plant P status based on availability of P. Based on a grain yield efficiency index they classified the

cultivars as P-efficient or P-inefficient. They concluded that shoot dry matter was more sensitive to P-deficiency and P use efficiency was higher under the low P treatment. Their study revealed that phosphorus uptake was significantly correlated with dry matter, P concentration and P efficiency ratio. Results of this study indicate that genetic differences in P-use efficiency exist among upland rice cultivars and may be exploited in breeding programs.

Ni *et al.* (1998), tested a set of five rice genotypes with different performance under P stress condition in both soil pot experiment and solution culture experiment under different P supply conditions to evaluate rice performance to P stress. The relative parameters (defined as a ratio of the parameters measured under P stress condition to the parameters measured under P sufficient condition) were used to evaluate the genotypic variation in tolerance to low soil P stress. They found that relative tillering ability, relative root number and relative root length, could be used to assess the genotypic tolerance to P stress in terms of P uptake ability and biomass production under P stress condition.

Ahmad *et al.* (2000), find that rice genotypes differed significantly in their growth response, in terms of paddy yield, relative reduction was ranged from none to 13%. The three genotypes, NIAB-6, IRRI-6 and K.BASMATHI were P responsive and productive and hence could be regarded as p-efficient rice genotypes.

Fageria and Santos (2002), conducted a greenhouse experiment to evaluate 12 genotypes of low land rice (*Oryza sativa* L.) using an Inceptisol. The P treatments were low (0 mg P kg⁻¹), medium (100 mg P kg⁻¹) and high (400 mg P kg⁻¹). They observed significant differences in genotype for P-use efficiency. On the basis of P-use efficiency, they classified the genotypes as (i) efficient and responsive, (ii) efficient and non-responsive, (iii) non-efficient and responsive and (iv) non-efficient and non-responsive. They suggested that practicality efficient and responsive and efficient and non-responsive genotypes are the most desirable ones. Among the 12 genotypes tested, none were found to be efficient and responsive in their study. Among the yield components, panicle length and harvest index were significantly affected by P levels and genotypes, significant interaction were observed between P level and genotypes.

Lim *et al.* (2003), observed the genotypic differences in three rice genotypes (Dasan, Sobi and Palawan) in the responses of APases to P deficiency in rice. The levels of shoot APase activity per protein were similar in the three genotypes. It is

significantly decreased with P deprivation that was longer than seven days. Root APase activity per protein was two to three folds higher in Dasan than in Sobi and Palawan. In all genotypes the APase activity increased in P-deficient plants, rather the increase was higher in Sobi and Palawan.

Wissuwa (2003), developed a plant growth model for the rice grown under highly P-deficient condition, to identify a primary mechanism to formulate hypotheses on pathways and genes causing genotypic differences in P uptake. Model simulations showed that small changes in root growth-related parameters had big effects on P uptake. It demonstrated that increasing root fineness or the internal efficiency for root dry matter production by 22% was sufficient to increase P uptake by a factor of three. Same effect could be achieved by a 33% increase in external root efficiency. However, the direct effect of increasing external root efficiency accounted for little over 10% of the 3-fold increase in P uptake. The remaining 90% was due to enhanced root growth as a result of higher P uptake per unit root size. Large genotypic differences in P uptake from a P-deficient soil can be caused by rather small changes in tolerance mechanisms. Such changes will be particularly difficult to detect for external efficiency because they are likely overshadowed by secondary root growth effects.

Akinrinde and Gaizer (2006), studied six varieties of rice in different soil P levels, and observed that varieties differed significantly in their growth, dry matter production and P-use efficiency. Based on their observation they categorize the varieties into efficient or non-efficient and responsive or non-responsive types.

Fageria and Filho (2007), conducted a field experiment in two consecutive years to determine dry-matter and grain yield, nutrient uptake and P-use efficiency of low land rice (*Oryza sativa* L.) grown on an acidic Inceptisol with application of 0, 131, 262, 393, 524, and 655 kg P ha⁻¹. They found significant and quadratic increase in Dry-matter yield of shoot and grain yield with P fertilization. They also found decrease of P use efficiencies (agronomic, physiological, agro-physiological, recovery, and utilization) with increasing P rates.

Du *et al.* (2008), conducted a research study to screen and identify the core rice collection of Yunnan landraces for high P efficiency. More than 500 rice accessions were planted in soil with low (6.26 mg kg⁻¹) and high P level (40 mg kg⁻¹) in the field, and low (0.02 mg kg⁻¹) and high P level (40 mg kg⁻¹) in pot culture. The relative grain yield per panicle was used as an index to screen and identify rice genotypes for

tolerance to low soil P. To avoid errors caused by single-screen indicators, weights of relative grain yield per panicle and relative traits closely correlated to relative grain yield per panicle were proposed as general screening index to screen for high P efficient germplasm lines. Among the core collection, 12 super high-P efficient germplasm lines such as Damogu, Xiyanggu, Haohanduo, Nuogu, Huagusanbaibang and Huangpinuo were identified as important materials for gene location and high P efficiency breeding. They also noticed P-deficiency tolerance of *indica* was more than that of *japonica* and P-deficiency tolerance of nuda rice was less than that of non-nuda rice.

Islam *et al.* (2008), conducted an experiment to clarify the effect of phosphorus on nutrient absorption characteristics of a *japonica* (Nipponbare) and *indica* (IR-28) rice varieties. The experiment was conducted under submerged condition in subsoil of an Andisol, which had a high capacity of phosphate fixation. Levels of phosphorus were 100, 200, 500 and 800 kg P₂O₅ ha⁻¹. They found that P content in rice plant at different growth stages increased progressively with an increase of P levels for both the varieties and with respect to P content, minimal varietal differences were observed between Nipponbare and IR-28.

Yaseen and Malhi (2009), conducted two field experiments to compare 15 wheat genotypes at two P levels (zero-P control or low P level-without application of P fertilizer on soil with 8 mg extractable P kg⁻¹, and adequate P level-with P fertilizer applied at 52 kg P ha⁻¹) for yield, P uptake, and P utilization efficiency. They observed substantial and significant differences among wheat genotypes for both grain and straw yields at both P levels. Relative reduction in grain yield due to P deficiency stress (PSF) ranged from none to 32%, indicating differential P requirements of genotypes. A wide range of P physiological efficiency index in their results indicated differential utilization of absorbed P by the genotypes for grain production. This indicated that wheat genotypes differed considerably in their P requirement for growth and also response to P application.

Wang *et al.* (2010), investigated tiller, floret, root biomass, P uptake and remobilization at two levels of P under field conditions in three winter wheat genotypes with different yield structures and P-efficiencies. Their results showed that P-efficient cultivars got higher yield and total P accumulation than P-inefficient at low-P, but not in normal P treatment. It was found that larger root rather than higher root activity was the determinant factor in efficient pre-anthesis P uptake in some entries and efficient post-anthesis P uptake in others. It was concluded that increasing wheat yield at low soil P

availability can be realized either by increasing ears per plant or increasing grains per ear through crop management or breeding.

Jakkeral and Kajjidoni (2011), investigated the correlation among twelve important characters in eighteen black gram genotypes under two Phosphorous levels at 45 DAS and at the time of harvesting. Analysis of data revealed that total P uptake significantly associated with plant height, root volume, root dry weight and shoot dry weight. Among root and shoot morphological traits they found positive significant association between root volume and plant height and between root surface area and root length, only under P deficient condition at 45 DAS. At the time of harvest they found, in both the P conditions, seed yield exhibited significant association with harvest index and seed P uptake, but total P uptake exhibited significant association with seed yield, only in P deficient condition. They suggested that selection of root volume at 45 DAS and seed yield at the time of harvest will be useful in direct and indirect selection of P efficient genotypes under P deficient condition.

Sarkar *et al.* (2011), screened 31 rice genotypes comprising of adopted upland and lowland lines in addition to aromatic and semi-dwarf high yielding cultivars. All the lines were grown under phosphorus-sufficient and phosphorus-deficient condition to check P-uptake ability. The lines were also genotyped for PCR-based markers, *Pup-1K42* and *Pup-1K29* which are linked to *PUP1* locus. Based on phenotypic and genotypic result three Bengal landraces, Bhutmuri, Gobindabhog and Radhunipagol found to be ideal donor parent for introgression of *Pup1* locus.

Krishnamurthy *et al.* (2014), evaluated twenty eight pre-release promising rice varieties and hybrids for grain yield and response to graded levels of applied phosphorus in a low soil-P fertility status calcareous vertisol (OlsenP:2.04ppmP). Among evaluated rice cultures, they observed four distinct patterns in grain yield response, eight rice cultures at P0-level, six rice cultures at medium P fertility level (20-30kgP₂O₅ha⁻¹) were exhibiting higher grain yield response; while five recorded higher grain yields and yield response only at higher P levels of 50-60kgP₂O₅ha⁻¹. Their results indicate the existence of genetic variability among the rice cultures for P use efficiency. They also suggest that the observed genetic variation can be utilized for breeding elite rice culture with superior grain yield stability under soil minimal nutrient availability, with minimal dependence on P fertilizer inputs.

Mukharjee *et al.* (2014), estimated the P acquisition efficiency from 108 rice genotypes to identify the best genotype suitable for soil like red and lateritic or acidic soils, with low soluble phosphate content. They observed that some genotypes accumulated almost equal amounts of phosphate even when they were grown on P-sufficient soil. In this study they have reported the presence as well as the expression of a rice gene, phosphate starvation tolerance locus (*PSTOL1*) in a set of selected genotypes. They also observed two of four genotypes did not show any detectable expression but carried the gene.

Panigrahy *et al.* (2014), reported the screening and characterization of M5 mutants derived from an ethyl methane sulfonate treated population of rice cv. Nagina 22 (N22) in low-P field (soil Olsen P 1.94–2.01 mg kg⁻¹; alkaline Vertisol; pH 7.94) for high yield. The study showed that seedling growth responses such as increase in root weight, root length, root/shoot weight, and dry weight in P-deficient medium can be taken as indices of low-P tolerance in mature plants in field. Total phosphorus content in seedlings showed an inverse relationship with total phosphorus content and low-P tolerance in mature plants in the field. But, phosphorus content in seeds and acid phosphatase activity in the seedling stage were positively correlated with survival and seed set in low-P field. In low-P field, plant height correlated most with yield per plant, and the number of productive tillers in mature plants was highly correlated with tiller number at vegetative stage.

Tian and Liao (2015), conducted pot experiment to study P effects on rice yield, dry matter and P accumulation and translocation in salt-alkaline soils. Plant dry weight and P content at heading and harvest stages of two contrasting saline-alkaline tolerant and sensitive rice varieties were examined under two saline-alkaline soils and five P supplements (P₀, P₅₀, P₁₀₀, P₁₅₀ and P₂₀₀ kg ha⁻¹). In severe saline-alkaline soil, they found dry weight, P accumulation and translocation, thousand grain weight, seed-setting rate and grain yield significantly decreased, but effectively increased with P application in some variety.

Aluwihare *et al.* (2016), screened the set of rice genotypes including landraces, old and new improved varieties for P deficiency tolerance in two major cropping seasons of Sri Lanka. They used the soil, which was collected from a plot cultivated with rice, without fertilizer application for past 40 years as the potting medium for greenhouse trials and two field trials were conducted in the same plots at RRDI, Sri Lanka. At the flowering stage, shoot dry weight, shoot P concentration, shoot P uptake

and P utilization efficiency were measured. All measured parameters were significantly ($P < 0.05$) differ between P_0 and P_{30} conditions. They used regression analysis between shoot dry weight and P utilization efficiency to categorize the rice genotypes into tolerant, moderately tolerant and sensitive to low soil P.

Vejchasarn *et al.* (2016), studied phenotypic variation and effect of phosphorus deficiency on root architectural, morphological and anatomical traits in 15 rice genotypes. They reported substantial genetic variation for all root traits investigated and demonstrate that low phosphorus availability significantly affect most of the traits and varied with the genotype.

Vandamme *et al.* (2016), assessed variation in grain P concentrations among rice genotypes across diverse environments and evaluate the implications for field P balances at various grain yield levels. They conducted Multi-location screening experiments at different sites across Africa and Asia, and determined the yield components and grain P concentrations at harvest. They observed genotypic variation in grain P concentration while considering differences in P supply and grain yield. The average grain P concentrations across genotypes varied almost 3-fold across the environments (1.4 to 3.9 mg g^{-1}).

Zhou *et al.* (2016), assessed the genetic variation on P use efficiency in soybean genotypes by comparing the yields and P acquisition potentials in the field and in hydroponic media containing low P treatment (0.05 mM L^{-1}) and high P treatment (0.25 mM L^{-1}). In the field trial, they found the models described the relationships between yield and seed P concentration ($R^2 = 0.85$), shoot P accumulation ($R^2 = 0.84$), Harvest Index ($R^2 = 0.82$). In the hydroponic trial, they found P-efficient genotypes under low P treatment showed significantly better plant growth, P accumulation and root to shoot ratio than P-inefficient genotypes. Similarly total root length, root surface area and root volume, rate of organic acid exudation and acid phosphatase activities of P-efficient genotypes were significantly greater than P-inefficient genotypes under low P treatment.

Vigueira *et al.* (2016), assessed *PSTOL1* polymorphism in geographically diverse sample of wild, domesticated and weedy rice and sequenced the gene in samples where it is present. Out of 282 screened plants, 160 carried *PSTOL1* and 122 were negative for all PCR genotyping reaction for *PSTOL1*. They have identified *PSTOL1* haplotype due to repeated evolution of loss-of function alleles through premature stops and framshift mutations. Assessment of plant growth under low and high-phosphorus at

greenhouse condition did not revealed significant association with *PSTOL1* genotypic variation for phosphorus deficiency.

Tian *et al.* (2017), conducted pot experiment to study P effects on rice yield, dry matter and P accumulation and translocation in salt-alkaline soils. Plant dry weight and P content at heading and harvest stages of two contrasting saline-alkaline tolerant and sensitive rice varieties were examined under two saline-alkaline (light versus severe) soils and five P supplements (P_0 , P_{50} , P_{100} , P_{150} and P_{200} kg ha⁻¹). Their results revealed that two rice varieties obtained relatively higher dry weight and P accumulation and translocation in P_0 condition. In severe saline-alkaline soil, they found dry weight, P accumulation and translocation, thousand grain weight, seed-setting rate and grain yield significantly decreased, but effectively increased with P application in some variety.

Wang *et al.* (2017), comprehensively investigated the relationship of P concentration and accumulation with yield formation and P use efficiency for grain production using 127 rice recombinant inbred lines (RILs) grown in a under low and high P conditions. P concentration and accumulation, P translocation and translocation efficiency, P harvest index, grain yield and grain yield components were investigated. Coefficients of variance in their study showed that the grain P concentration was considerably conserved, whereas the straw P concentration was relatively variable among inbred lines. Study revealed that in comparison with grain P, the straw P on tributes more total P accumulation at maturity. The results show that low straw P concentrations may simultaneously improve P use efficiency for grain production and grain yield by enhancing P translocation into grain, thus reducing the need for P fertilizer application.

Vellaikumar and Malarvizhi (2017), studied a set of 120 rice genotypes (composed of upland cultivars and landraces) in low P soils with three different P fertilizer dose inputs (P_0 , P_{25} and P_{50} kg ha⁻¹ P) to compare P uptake efficiency. Their results showed that, among the 120 genotypes, 12.5% genotypes produced higher yield under low soil P condition. Further, screening of these genotypes presence of *Pup1* gene by PCR amplification by two *Pup1* associated insertion and deletion (InDel) markers viz, Pup1-K46 and Pup1-K52. The screening confirmed the presence of *Pup1* gene in more than 90% of these genotypes.

Chankaew *et al.* (2019), screened 168, rice varieties including Thai indigenous upland rice germplasm under hydrponic experiment with phosphorus sufficient and

phosphorus deficient condition to study variation for phosphorus deficiency tolerance and validate *Pup1* gene linked markers. Total eleven upland rice accessions were classified as phosphorus deficiency tolerant out of which two lines ULR183 and ULR213 showed high tolerance without *Pup1* linked gene product that can serve as source of exploration of novel genes for phosphorus deficiency tolerance.

2.5 Marker assisted selection and importance of breeding for low soil phosphorus tolerance

Deficiency in phosphorus (P) can severely limit rice yields. It was estimated that approximately 20-30% of the applied phosphate are available for plant, while around 80-90% of P is transformed to other forms that are not easily available to plants, eventually lost in the environment hence, causing water eutrophication (Schindler *et al.*, 2016). Developing cultivars with tolerance to P deficiency may represent a more sustainable solution than sole reliance on fertilizer application. To better answer the P crisis in rice farming, the development of P efficient rice genotypes, which are adapted to low P soils, would be a promising solution (Cordell *et al.*, 2009; Rose and Wissuwa, 2012). In many studies, screening of rice genotypes for phosphorous deficiency tolerance has been conducted successfully and Phosphorous efficient genotypes can be used as parents in the breeding programs (Wissuwa *et al.*, 1998; Chin *et al.*, 2011). Hence, breeding for low soil P is one of the best strategies to achieve varieties with low soil phosphorous tolerance.

Phosphorus uptake 1 (Pup1) is a major quantitative trait locus (QTL) located on rice chromosome 12 that is associated with tolerance of phosphorus (P) deficiency in soil (Wissuwa *et al.*, 1998; Wissuwa *et al.*, 2002). Kasalath, the *Pup1* donor variety, was initially identified in a screening of thirty diverse rice genotypes in a P-deficient soil in Japan under rainfed conditions. Subsequently, phenotypic data derived from Nipponbare contrasting near-isogenic lines (NILs) with and without the QTL showed that *Pup1*, increases P uptake (Wissuwa and Ae 2001; Wissuwa *et al.*, 2002) and confers a significant yield advantage (2-4 fold higher grain weight plant⁻¹) in pot experiments using different P-deficient soil types and environments (Chin *et al.*, 2010). To identify the genes associated with *Pup1*, the genomic region had been sequenced in the donor variety Kasalath, revealing a complex locus with large insertion-deletions (INDELs) and overall low conservation compared with the corresponding genomic regions in the Nipponbare (Chin *et al.*, 2010).

Chin *et al.* (2011), used the gene models predicted in the *Pup1* region in the donor variety Kasalath to develop gene-based molecular markers that are evenly distributed over the fine-mapped 278-kb QTL region. To validate the gene models and optimize the markers, gene expression analyses and partial allelic sequencing were conducted. And a core set of *Pup1* markers has been defined, and sequence polymorphisms suitable for single nucleotide polymorphism marker development for high-throughput genotyping were identified. By following a marker-assisted backcrossing approach, *Pup1* was introgressed into two irrigated rice varieties and three Indonesian upland varieties. They were also conducted the first phenotypic evaluations of the introgression lines and suggest that *Pup1* is effective in different genetic backgrounds and environments and it has the potential to significantly enhance grain yield under field conditions.

Chithrameenal *et al.* (2018), have made an attempt to develop popular local rice varieties of tamilnadu, ASD 16 and ADT43 for bacterial blight, blast and low soil P tolerance. They produced backcross inbred lines for bacterial blight and blast and then carried out introgression of *OsPSTOL1* for low soil P tolerance. The lines with better tolerance for low soil P tolerance produced enhanced root characters.

Anila *et al.* (2018), used marker-assisted backcrossing breeding (MABB) for introgression of *Pup1* a QTL from Swarna (low soil P tolerant) into MTU1010 (low soil P susceptible tolerant) to improve it for low soil P tolerance. Foreground selection of backcross plants carried out with the help of functional marker K20-2 and K46-1. Total 85, markers used for analysis of background recovery. Five introgressed lines confirmed for *Pup1* and maximum recurrent parent genome recovery were further screened in low soil P and normal soil P plot. The introgressed lines with *Pup1* showed higher yield (>390%) as compared to recurrent parent MTU1010.

By looking into the previous studies and reports it's clear that P deficiency tolerance is an important area in rice breeding. The identified QTL *Pup1* is promising and can be utilized in the improvement of rice genotypes against low soil P sensitivity. Presence of huge variability in rice germplasm gives a hint on presence of novel variants for P use efficiency. Further, introgression of low soil P tolerance by conventional backcross method is time and resource consuming but marker assisted selection paved the way in breeding for low soil P tolerance.

2.6 Molecular markers used for molecular breeding

The fundamental basis of plant breeding is the selection of specific plants with desirable traits. Conventional selection typically involves morphological assessment of different breeding population for identification of superior plants for one or more biotic and abiotic traits in field or glass house conditions or with grain quality related traits. However, environmental influence on the trait expression makes the selection process confounding. Utility of DNA markers were constantly improved to provide easy, fast and automated assistance since their development, which facilitated development of marker based gene tags, genetic maps, map based cloning, phylogenetic analysis and marker-assisted selection (Collard *et al.*, 2008). DNA marker helps in the study of genetic basis of complex phenotypes such as epistasis, pleiotropy and genotype x environment interaction, understanding of plant genetic diversity and mapping of genomic regions that contribute to trait variation (Toojinda *et al.*, 1998; Dwivedi *et al.*, 2007). The putative gene affecting the trait of interest was identified using statistical associations between marker and the trait (Paterson *et al.*, 1991; Cheema *et al.*, 2008; Sama *et al.*, 2012). Another application of molecular markers is Marker assisted selection (MAS) is very effective in identifying resistance or tolerance genes transferred to different genetic backgrounds at an early stage with greater accuracy (Gu *et al.*, 2005). Various types of molecular markers used in molecular breeding program are as follows:

2.6.1 Restriction fragment length polymorphisms (RFLPs)

RFLPs are generated by digesting the DNA with the help of special kind of enzymes called restriction endonucleases (RE), which cut the DNA at or near the restriction site. The restriction fragment so generated is then hybridized with labeled DNA probe, then visualized to detect target DNA. This is the first class of DNA markers (Botstein *et al.*, 1980) that are used in various crop species including rice for mapping QTL or genes for various biotic and abiotic stresses (Lin *et al.*, 1996).

2.6.2 Amplified fragment length polymorphisms (AFLPs)

The AFLPs is the technique which combines basic features of both hybridization based RFLP and the PCR based approaches. The procedure to generate AFLPs is based on the selective PCR amplification of the restriction fragments from a total digest of genomic DNA (Zabeau and Vos, 1993; Vos *et al.*, 1995). In this technique, specific double stranded DNA adapters are ligated to the DNA restriction fragments so that the sequence of adapters and the adjacent restriction sites serve as primer binding sites. Like RAPD, AFLPs are dominant markers and hence could not be able to distinguish

homozygous and heterozygous condition and not so popular among molecular breeders. In case of rice, AFLP used as a DNA fingerprinting technique to study genetic diversity (Zhu *et al.*, 1998; Federici *et al.*, 2001)

2.6.3 Diversity Arrays Technology (DArT)

Microarray-based markers such as Diversity Arrays Technology (DArT) have become the genetic markers of choice for construction of high-density maps, quantitative trait loci (QTL) mapping and genetic diversity analysis based on their efficiency and low cost. The DArT markers revealed genetic relationships among the accessions consistent with the available information and systematic classification. Phung *et al.* (2014), has genotyped the panel of Vietnamese rice varieties using Diversity Array Technology (DArT) markers to constitute a highly valuable resource for mining new alleles within Vietnamese genetic resources. Genome-Wide Association mapping of root traits in a Japonica Rice Panel using DArT markers used to study association mapping in a japonica panel for various root related traits (Courtois *et al.*, 2013). In another study, Mogga *et al.* (2018), demonstrated that DArT seq markers were useful genomic resources for genome-wide association studies of rice grain quality traits to accelerate rice breeding program.

2.6.4 Random amplified polymorphic DNA (RAPD)

RAPD is the simplest example of PCR based marker that involves the use of 10 base pair random primers (Williams *et al.*, 1990). RAPD could not become very popular in gene mapping studies due to their inability to distinguish between homozygous and heterozygous individuals, and their unreliable reproducibility. RAPD have been widely used for genetic diversity studies, identification of cytoplasmic male sterile (CMS) lines including rice (Vierling and Nguyen, 1992; Fernandez *et al.*, 2002; Souframanien *et al.*, 2003; Rahman *et al.*, 2006)

2.6.5 Sequence characterized amplified regions (SCARs)

These markers are applied for amplification of specific bands. SCARs are derived by cloning and sequencing the ends of RAPD/AFLP markers that appeared to be diagnostic for specific purpose (Paran and Michelmore, 1993). As they can detect only a single locus, hence are more reproducible and beneficial than RAPDs. SCAR markers successfully used in rice to identify disease related genes and to distinguish rice

variety with similar morphological characters (Naqvi and Chattoo, 1996; Saengprajak, 2012).

2.6.6 Inter simple sequence repeats (ISSRs)

This class of markers involves the amplification of the DNA segment present at amplifiable distances between two identical microsatellites oriented in the opposite direction. Here the microsatellite repeats are used as primers targeting multiple genomic loci to amplify ISSR of different sizes. These are mostly dominant markers, though occasionally a few of them exhibit co-dominance. ISSR amplification proved to be a valuable method for determining genetic variability among rice varieties and for rapidly identifying cultivars (Blair *et al.*, 1999; Alhasnawi *et al.*, 2015; Al-Turki and Basahi, 2015).

2.6.7 Cleaved Amplified Polymorphic Sequences (CAPS) marker

CAPS (Cleaved Amplified Polymorphic Sequences) represent a distinct group of well-known markers that are successfully applied, especially in the field of plant biology. The principle of CAPS based on at least three consequent steps: (1) PCR with specific primers; (2) Digestion of amplification fragments (amplicons) with restriction enzymes (endonucleases); and (3) Separation of digestion products in an agarose gel. In fact, CAPS markers combine widely used PCR techniques with classical methods of Restriction Fragment Length Polymorphism (RFLP), only it is based on the amplification of small fragments of DNA instead of the entire genome (Heubl, 2010; Lu *et al.*, 2010).

2.6.8 Single nucleotide polymorphisms (SNPs)

SNPs markers are based on sequence differences at single base pair (bp) positions in genomes. SNPs can be categorized according to nucleotide substitutions either as transitions (C/T or G/A) or transversions (C/G, A/T, C/A or T/G). Single nucleotide exchanges in genomes are numerous; typically, SNP frequencies are in a range of one SNP every 100-300 bp in plants (Xu, 2010) therefore SNP markers provide a great marker density. SNPs are co-dominant markers, often linked to genes and present in the simplest/ ultimate form for polymorphism, and thus they potential genetic markers in the genetic study and breeding. The various application of SNP marker include construction of genetic maps, cloning of QTLs, to perform high-throughput

genotyping, dissection of complex traits (Feltus *et al.*, 2004; Kumar *et al.*, 2012; Huang *et al.*, 2014; Yu *et al.*, 2014; Chen *et al.*, 2014). Various recently developed SNP array has been used for study of diversity and genome wide association as well as identification on QTLs and genes in rice and other crops (Zhao *et al.*, 2011; Jewel *et al.*, 2019).

2.6.9 Simple Sequence Repeats (SSRs) Markers

Simple Sequence Repeat (SSR) / microsatellite markers are simple, tandem repeats of di- to tetra-nucleotide sequence motifs flanked by unique sequences. They are valuable as genetic markers because they are co-dominant, detect high levels of allelic diversity, and easily and economically assayed by PCR techniques. Microsatellites consist of multiple copies of tandemly arranged simple sequence repeats (SSRs) that range in size from 1 to 6 base pairs (Tautz *et al.*, 1986; Litt and Luty, 1989). SSR markers can distinguish different alleles of a locus that make it more powerful. A further advantage is the fact that abundant occurrence of microsatellites in plants, high level of information, abundance and uniform distribution in the genome combined with reliable amplification via PCR (Powell *et al.*, 1996; McCouch *et al.*, 2002). However, it suffers from some of the limitations such as the requirement of nucleotide information for primer design, labor-intensive marker development process and high start-up costs for automated detections. In microsatellite analysis, sequence information of the regions flanking the repeats is used for creating locus-specific PCR primer pairs. The resulting amplification products are separated on gel and visualized.

Using publically available BAC and PAC clones, more than 200 validated SSRs were released in 2002 (McCouch *et al.*, 2002). This was soon followed by 18828 Class I (di-, tri-, tetra-repeats) SSRs that were released after the completion of the Nipponbare genome sequence in 2005 (Matsumoto *et al.*, 2005). The extremely high density of SSRs (~51 SSRs per Mb) has provided a considerable “tool kit” for map construction and MAS for numerous applications. It has been observed that genetically mapped microsatellite markers cover the whole rice genome with at least one microsatellite for every 157 kb (<1 cM) (Chen *et al.*, 1997; McCouch *et al.*, 2002). Provan *et al.* (1997), using SSRs, detected intra and inter cultivar polymorphism between the cultivated and wild rice. Sebastian *et al.* (1998), studied the molecular diversity of Philippine landraces and cultivars and found that diversity is more in land races compared to modern cultivars. Application of SSR markers in the estimation of diversity among cultivars,

inbreds and parental lines of rice, tagging and mapping of a major QTL and genes have been reported in rice (Huang *et al.*, 1997; Zhang *et al.*, 1998; Enoki *et al.*, 2002; Cordeiro *et al.*, 2002; Chen *et al.*, 2003; Xu *et al.*, 2004; Neeraja *et al.*, 2007; Cheema *et al.*, 2008).

2.7 Mapping populations and approaches for QTL mapping

Molecular markers are of great value in applying genetic technologies to crop improvement. One of the most important applications of DNA markers and molecular linkage maps is to dissect the genetic variation of quantitative traits into individual Mendelian factors through QTL mapping analyses. For genetic mapping studies aimed at the preparation of framework genetic maps, the size of the mapping populations generally varies from 50 to 250 segregating individuals/ lines (Mohan *et al.*, 1997). Availability of segregating mapping population, derived from a cross between two parents, which differ for one or more traits of interest, is one of the pre-requisites for gene tagging and QTL mapping. Molecular markers have been extensively used for construction of genetic maps utilizing mapping populations which could be F_2 , backcross population, double haploids (DH) and recombinant inbred lines (RILs) (Kanbar *et al.*, 2002; Kamoshita *et al.*, 2002; Price *et al.*, 2002; Nguyen *et al.*, 2004; Horii *et al.*, 2006). For QTL studies it is important that the population must be evaluated phenotypically prior to the QTL analysis. Construction of linkage map involves genotyping of segregation data of each polymorphic marker on each individual of the mapping population and subsequent analysis of this segregation data using appropriate software like MAPMAKER (Lander and Botstein, 1989) and JOINMAP (Stam, 1993), ICimapping (Meng *et al.*, 2015) and Win QTL cartographer (Wang *et al.*, 2005a). Generally, a LOD (logarithm of odds) value of 3 is used to construct linkage map. A LOD value of 3 indicates that linkage between markers is 1000 times more likely than no linkage (null hypothesis). The distances between different markers are designated in centimorgans (cM) which is obtained by converting recombination frequencies into marker distances (cM) with suitable mapping functions like Haldane (1919) and Kosambi (1944).

Backcross (BC) populations and near-isogenic lines (NILs), derived by crossing the F_1 hybrid to one of the parents are simple and easy to develop (Ishimaru, 2003; Garcia-Oliveria *et al.*, 2008; Jewel *et al.*, 2019). The disadvantage of using F_2 /BC populations is that they do not represent true breeding lines. So, they cannot be

evaluated in repeated experiments. The use of advanced backcross population in QTL analysis (AB-QTL analysis) has the advantage of simultaneous identification and selection of new and beneficial QTL alleles of exotic ancestors and their introduction into elite gene pools (Tanksley and Nelson, 1996; Yoon *et al.*, 2006). Yet another type of mapping population is the recombinant inbred line (RIL) population which is a series of homozygous lines derived from the inbreeding and single seed propagation of separate individual plants of an F_2 population, each containing a unique combination of chromosomal segments from the original parental genotypes (Qu *et al.*, 2008). Each RIL of a mapping population is homozygous for trait and may be multiplied without segregation. The RILs thus constitute immortal mapping populations, which are most suitable for repeated evaluation under different environments. The only disadvantage of the RIL mapping population is the long duration required for their development. RIL populations are the most commonly used mapping populations for QTL analysis in crop plants.

In QTL mapping, genes controlling genetic variation of quantitative traits in segregating populations are resolved into individual Mendelian factors by detecting marker-trait associations. Another major purpose of QTL mapping is to identify DNA markers diagnostic for phenotypes of interest so that marker aided selection (MAS) can be used to efficiently manipulate progenies carrying alleles for target traits grown under non-target environments (Yoon *et al.*, 2006). QTL analysis provide mainly major and minor QTLs, such that minor QTL is one which contribute to overall phenotypic variation in the order of 25% or less (Price, 2006). QTL mapping are based on three broad classes: regression, maximum likelihood and Bayesian models. Over the years, several statistical methods have been developed for detection of QTL. Broadly these includes;

In single marker analysis (SMA), the marker-trait association is worked out through the regression of phenotypic values on marker genotype. The simplest single marker analysis identifies QTL based on the difference between the mean phenotypic value of different marker groups, but cannot separate recombination fraction and QTL effect. However, this approach is of limited use in locating the chromosomal segment that contains a QTL. The method also underestimates the effect of the QTL. The farther the QTL is from the marker, the weaker is the effect measured.

In the interval mapping (IM) approach (Lander and Botstein, 1989), QTLs are located within chromosomal intervals, defined by the flanking markers. Interval

mapping (IM) is based on maximum likelihood parameter estimation and provides a likelihood ratio test for QTL position and effects. The major disadvantage of IM is that the estimate of locations and effects of QTL may be biased when QTL are linked. The technique involves screening of each interval at regular genetic distances to assess the probability of the presence of a QTL in each such screened segment of an interval that is defined by a pair of flanking markers.

Combining of IM with regression analysis (Jansen, 1993; Zeng, 1994) is known as ‘Composite Interval Mapping’ (CIM), which assesses the probability of the presence of a QTL, while controlling the effect of other QTL in the background and thus increases the precision of QTL detection. However, even in this approach, one examines the presence of a single QTL at a time, so that there is no scope for examining epistatic interactions. LOD peaks for significant QTL were used to position QTL on the linkage map. Gene action and percent variation attributable to individual QTL were estimated at peaks.

Kao *et al.* (1999), extended this approach to a multiple QTL model for mapping multiple QTL at a time and named it ‘Multiple Interval Mapping’ (MIM). It makes use of multiple intervals simultaneously to detect multiple putative QTL, so that epistatic interactions can also be examined. Therefore, when compared with IM and CIM, MIM is not only more powerful and precise in detecting QTL, but also allows detection of epistatic interactions.

2.8 Genes/QTL and associated markers identified for low P tolerance

Various studies has carried with the help of different mapping population to map the gene or QTLs involved in low soil P tolerance (Table 2.1). Wissuwa *et al.* (1998), made a cross of the intolerant *japonica* cultivar, Nipponbare “with the tolerant *indica* landrace, Kasalath” and identified Quantitative trait loci (QTLs) for P deficiency tolerance. To identify putative QTLs for P-deficiency tolerance in rice, they used 98 backcross inbred lines derived from a *japonica* × *indica* cross and genotyped at 245 RFLP marker loci. Using Composite Interval Mapping (CIM) method, a major QTL for P uptake was mapped to a 13.2 cM interval on the long arm of chromosome 12, flanked by markers C443-G2140. The position was estimated to be at 54.5 cM, a 3 cM distance from marker C443. Additional minor QTLs were found on chromosomes 2, 6, and 10. For three of the four QTLs, including the major one, the positive allele came from

Table 2.1: List of QTLs associated and molecular markers identified for low soil P condition from diverse mapping populations in rice

S. No	QTLs associated traits	Interval markers	Mapping population	Type	Chr	LOD	PV	Reference
1	PUP	G227-C365	Nipponbare × Kasalath	BILs	2	2.82	5.8	Wissuwaet <i>al.</i> , , 1998
		C498-R1954			6	3.52	9.8	
		R1629-R2447			10	4.7	7.7	
		G2140-C443			12	10.74	27.9	
		G227-C365			2	2.82	5.8	
		C498-R1954			6	3.52	9.8	
		BIN1348-BIN1349	Zhenshan 97 × Minghui 63	RILs	10	-	5.5	Wang <i>et al.</i> , 2014
		BIN46-BIN47			1	-	4.7	
		S14025-S13126	Nipponbare × NIL-C443	NILs	12	16.6	78.9	Wissuwaet <i>al.</i> , 2002
		S14025-S13126	Nipponbare × NIL-C443	F3	12	11.5	28.1	
2	PUE	G227-C365	Nipponbare × Kasalath	BILs	2	5.22	9.8	Wissuwaet <i>al.</i> , 1998
		C946-R1854			4	4.65	9.4	
		G2140-C443			12	6.57	19.1	
		G227-C365			2	11.4	32.1	
		CT221-C223	ZYQ8 × JX17	DHs	10	2.16	10.2	Fenget <i>al.</i> , 2000
3	PUEg	BIN302-BIN303	Zhenshan 97 × Minghui 63	RILs	2	-	13.4	Wang <i>et al.</i> , 2014

		BIN1395-BIN1396			11	-	4.4	
		BIN946-BIN947			6	-	4.1	
4	RRDW	RM410-RM553	Yuefu × IRAT109	ILs	9	-	5.15	Li <i>et al.</i> , 2009
		G200-C235	ZYQ8 × JX17	DHs	6	5.12	20.5	Fenget <i>et al.</i> , 2000
		RM229-RM5349	Yuefu × IRAT109	ILs	11	>3.0	9.92	Li <i>et al.</i> , 2009
		RM290	Shuhui 527 × Yetuozai Minghui 86 × Yetuozai	ILs	2	-	8.5	Chao <i>et al.</i> , 2015
		RM576			1	-	8.2	
		RM142			4	-	11.5	
		RM20A			12	-	7.7	
		RM511			12	-	14.1	
		RM410 RM553	Yuefu × IRAT109	ILs	9	>3.0	5.15	Li <i>et al.</i> , 2009
		RG9-RG241	IR20 × IR55178-3B-9-3	RILs	12	12.05	47	Ni <i>et al.</i> , 1998
		E4/M8-3&E1/M7-7			6	5.8	32.3	
		RM5745-RM136	Xieqingzao B x Zhonghui9308	RILs	6	2.94	8.44	Zhang <i>et al.</i> , 2017
		RM311-RM6142			10	3.36	10.8	
		RM286-RM4			11	3.49	10.54	
		InD36	Xieqingzao B x Zhonghui9308	CSSLs	3	2.00	10.82	Anis <i>et al.</i> , 2018
		RM3638			5	2.37	12.66	
		InD133			10	2.18	12.52	
		RM10049- RM10116	<i>O. sativa</i> L. X <i>O.</i> <i>rufipogon</i> Giff	BILs	1	2.85	5.98	Luo <i>et al.</i> , 2017
		RM282-RM6266			3	3.74	24.49	
		RM481-IN7-4.5			7	2.89	10.39	

		RM3452-RM447			8	2.53	6.13	
		IN9-3-RM444			9	3.92	22.93	
5	RTDW	RM259-RM576	Yuefu $\times$ IRAT109	ILs	1	-	-	Li <i>et al.</i> , 2009
		G200-C235	ZYQ8 $\times$ JX17	DHs	6	6.6	24.9	Fenget <i>et al.</i> , 2000
		RM283	Shuhui 527 $\times$ Yetuozai Minghui 86 $\times$ Yetuozai	ILs	1	-	16.5	Chao <i>et al.</i> , 2015
		RM576			1	-	8.2	
		RM16			3	-	10.6	
		RM514			3	-	8.2	
		RM335			4	-		
		RM219			9	-	8.2	
		RM219			9	-	11	
		RM155			12	-		
		RM511			12	-	8	
		RM212-RM315			1	2.72	8.06	Luoet <i>et al.</i> , 2017
		RM282-RM6266			3	4.21	29.41	
		RM20B-RM286			11	3.32	7.15	
		RM1233-RM224			11	3.48	8.66	
6	RSDW	G200-C235	ZYQ8 $\times$ JX17	DHs	6	6.79		
		RM283	Shuhui 527 $\times$ Yetuozai Minghui 86 $\times$ Yetuozai	ILs	1	-	8.5	Chao <i>et al.</i> , 2015
		RM16			3	-	9.7	
		RM574			5	-	13	
		RM26			5	-		

		RM589			6	-	25.2	Fenget <i>al.</i> , 2000
		RM481			7	-	14.6	Chao et al., 2015
		RM155	Shuhui 527 × Yetuozai Minghui 86 × Yetuozai	ILs	12	-	8.5	
		RM511			12	-	8.7	
		RM307-RM5953	Xieqingzao B x Zhonghui9308	RILs	4	5.23	15.17	Zhnaget <i>al.</i> ,2017
		RM564-RM3207			6	2.78	7.43	
		RM20B-RM286			11	2.89	6.15	
		RM1233-RM224			11	3.56	7.79	
7	RRL	RM521	Shuhui 527 × Yetuozai Minghui 86 × Yetuozai	ILs	2	-	14.5	Chao <i>et al.</i> , 2015
		RM3295			5	-	16.6	
		RM510			6	-	12.6	
		RM3307			6	-	14.4	
		RM5926-RM224	Yuefu × IRAT109	ILs	11	-		
		RM322	OM2395 × AS996	BC2F3	2	>3.0	9.19	Nguyen and Bui, 2006
		RM3670-RM2	Xieqingzao B x Zhonghui9308	RILs	7	4.88	24.92	Zhang <i>et al.</i> ,2017
		RM320-RM182			7	3.33	10.49	
		RM167-RM5704			11	2.50	13.45	
		RM212-RM315	<i>O. sativa</i> L. X <i>O. rufipogon</i> Giff	BILs	1	4.45	9.32	Luoet <i>al.</i> , 2017
		RM546-RM517			3	2.67	8.88	
		InD90	Xieqingzao B x Zhonghui9308	CSSLs	6	2.31	16.07	Anis <i>et al.</i> , 2018
		RM5926-RM224	Yuefu × IRAT109	ILs	11	>3.0	8.68	Li <i>et al.</i> , 2009
8	RWRSR	RM3688	Shuhui 527 × Yetuozai	ILs	2	-	10.80	Chao <i>et al.</i> , 2015

			Minghui 86 × Yetuozai					
		RM514			3	-	8.2	
		RM538			5	-	8	
		RM589			6	-	10.3	
		RM510			6	-	8.8	
		RM219			9	-	8	
		RM184			10	-	13.4	
		RM287			11	-	7.9	
		RM155			12	-	11.5	
9	PNPP	RM283	Shuhui 527 × Yetuozai Minghui 86 × Yetuozai	ILs	1	-	16.50	Chao <i>et al.</i> , 2015
		RM169			5	-	8.5	
		RM26			5	-	8.7	
		RM536			11	-	13	
10	PUEstr	BIN302-BIN303	Zhenshan 97 × Minghui 63	RILs	2	-	6.3	Wang <i>et al.</i> , 2014
		BIN177-BIN178			1	-	4.9	
11	PT	BIN1130-BIN1131	Zhenshan 97 × Minghui 63	RILs	8	-	5	Wang <i>et al.</i> , 2014
		BIN709-BIN710			5	-	4.9	
12	PTE	BIN310-BIN311	Zhenshan 97 × Minghui 63	RILs	2	-	9.9	Wang <i>et al.</i> , 2014
		BIN1131-BIN1132			8	-	7.2	
		BIN33-BIN34			1	-	6.4	
13	SPF	RM569-RM267	IRAT109 X Yuefu	DH	5	3.89	16.48	Ping <i>et al.</i> ,2008
		RM341-RM208			2	3.62	11.50	
		RM231-RM175			3	3.13	6.79	
		RM314-RM253			6	3.33	5.86	

		OSR1-RM202			11	4.91	14.32	
		RM9	Shuhui 527 × Yetuozai Minghui 86 × Yetuozai	ILs	1	-	6.8	Chao <i>et al.</i> , 2015
		RM335			4	-	11	
		RM164			5	-	10	
		RM26			5	-	8.7	
		RM5463			6	-	9.8	
		RM254			11	-	9.7	
14	TGW	RM2247-C746	IRAT109 X Yuefu	DH	3	5.13	14.38	Ping <i>et al.</i> ,2008
		RM335	Shuhui 527 × Yetuozai Minghui 86 × Yetuozai	ILs	4	-	11.00	Chao <i>et al.</i> , 2015
		RM164			5	-	10	
		RM5463			6	-	9.8	
		RM481			7	-	9.2	
		RM219			9	-	8	
		RM335			4	-	12.3	
		RM5463			6	-	13.7	
		RM481			7	-	7.7	
		RM219			9	-	13.7	
15	RSL	RM247	OM2395 × AS996	BC2F3	12	>3.0	11.21	Nguyen and Bui, 2006
		RM316			9	>3.0	11.56	
		RM291			5	>3.0	11.67	
		RM235			12	>3.0	11.01	
		InD90	Xieqingzao B x Zhonghui9308	CSSLs	6	3.02	16.94	Anis <i>et al.</i> , 2018
		RM337				d		

16	TNPP	R374-C335	Nipponbare × Kasalath	BILs	4	4.37	9.8	Wissuwaet <i>et al.</i> , 1998
		C901-G1406			12	3.9	9.5	
		G2140-C443			12	7.87	20.6	
		OSR22-RM202	IRAT109 X Yuefu	DH	7	4.08	17.82	Ping <i>et al.</i> ,2008
		RM216-C1369			10	4.32	17.35	
		RM270-RM235			12	3.12	18.43	
17	REP	RM439	Nipponbare × Kasalath	CSSLs	6	95.2	54.5	Shimizu <i>et al.</i> , 2008
		RM5814			6	95.2	54.5	
		RM7555-RM3343			6	3.9	3.6	
		RM30	Gimbozu × Kasalath	F8	6	4.5	19.9	Shimizu <i>et al.</i> , 2004
18	PDW	C1488-C63	Nipponbare × Kasalath	BILs	3	3.08	6.4	Wissuwaet <i>et al.</i> , 1998
		C191-C498			6	4.71	9.7	
		G2140-C443			12	10.5	26.5	
19	RTA	RG9-RG241	IR20 × IR55178-3B-9-3	RILs	12	16.51	42.9	Ni <i>et al.</i> , 1998
		E3/M8-3-E1/M7-7			6	7.82	27.8	
		RM311-RM6142	Xieqingzao B x Zhonghui9308	RILs	10	3.66	12.41	Zhang <i>et al.</i> ,2017
		RM206-RM1233			11	3.30	10.05	
20	RRN	RM1112-RM1272	Yuefu × IRAT109	ILs	4	>3.0	-	Li <i>et al.</i> , 2009
		RM2334-RM3166			3	>3.0	9.61	
		RM2334-RM3166	Yuefu × IRAT109	ILs	3	-	9.49	
		RM3638	Xieqingzao B x Zhonghui9308	CSSLs	5	2.21	12.71	Anis <i>et al.</i> , 2018

		RM546-RM517	<i>O. sativa</i> L. X <i>O. rufipogon</i> Giff	BILs	3	2.86	6.07	Luo <i>et al.</i> , 2017
21	BIOM	BIN1342-BIN1343	Zhenshan 97 × Minghui 63	RILs	10	-	12.3	Wang <i>et al.</i> , 2014
		BIN31-BIN32			1	-	8.6	
		BIN1008-BIN1009			7	-	7.8	
23	HI	BIN59-BIN60	Zhenshan 97 × Minghui 63	RILs	1	-	9.7	Wang <i>et al.</i> , 2014
		BIN1398-BIN1399			11	-	7.4	
24	PHI	BIN59-BIN60	Zhenshan 97 × Minghui 63	RILs	1	-	15.8	Wang <i>et al.</i> , 2014
		BIN310-BIN311			2	-	20	
		BIN1392-BIN1393			11	-	4.4	
25	RPH	RM7269-RM5314	Yuefu × IRAT109	ILs	6	-	-	Li <i>et al.</i> , 2009
		RM6911-RM492	Yuefu × IRAT109	ILs	2	>3.0	11	
26	RER	GGCACC(247A, 293B)H	Gimbozu × Kasalath	F8	6	4.55	20.3	Shimizu <i>et al.</i> , 2004
27	RPUC	G200-C235	ZYQ8 × JX17	DHs	6	2.8	11.9	Fenget <i>et al.</i> , 2000
28	PCS	GCTACA474A	Gimbozu × Kasalath	F8	6	6.46	24.6	Shimizu <i>et al.</i> , 2004
29	RRV	RM6911-RM492	Yuefu × IRAT109	ILs	2	-	-	Li <i>et al.</i> , 2009
30	GY	RM200	Shuhui 527 × Yetuozai Minghui 86 × Yetuozai	ILs	1	-	16.2	Chao <i>et al.</i> , 2015
		RM208-RM48	IRAT109 X Yuefu	DH	2	3.68	4.77	Ping <i>et al.</i> , 2008
		C746-R3226			3	4.14	11.43	
		G2028-RM3226			6	4.53	13.55	
		OSR22-RM11			7	3.83	11.23	
31	RRRS	RM307-RM5953	Xieqingzao B x Zhonghui9308	RILs	4	3.27	9.92	Zhnaget <i>et al.</i> , 2017
		RM5953-RM3317						

32	RSFW	RM212-RM315	<i>O. sativa</i> L. X <i>O. rufipogon</i> Giff	BILs	1	5.22	8.86	Luoet al., 2017
		RM208-RM286			11	3.22	6.75	
		RM1233-RM224			11	5.99	13.75	
		RM254-RM1233			11	3.31	32.05	
33	RRFW	RM29-RM475	<i>O. sativa</i> L. X <i>O. rufipogon</i> Giff	BILs	2	4.24	37.07	Luoet al., 2017
		RM6266-RM16			3	3.90	37.32	
		IN9-3-RM444			9	3.81	31.88	
		RM254-RM1233			11	3.52	34.70	
34	GN	G51-RM231	IRAT109 X Yuefu	DH	3	3.02	2.65	Ping et al.,2008
		C107-C734			4	3.58	5.62	
		OSR21-G2028			6	3.91	20.78	
		R1488-C1008			7	4.38	13.47	
37	Psi-AAP	E4-M3-11/E5-M6-2	IR20 X IR55178-3B-9-3	RILs	1	4.12	19	Hu et al.,2001
		E3-M8-3/E1-M7-7			6	3.96	20	
		E3-M6-4			12	3.09	15	

PUP- Total P uptake; **PUE**- P use efficiency;**PUEg**- Grain P use efficiency; **RRDW**- Relative root dry weight; **RTDW** -Relative total dry weight; RSDW- Relative shoot dryweight; **RRL**- Relative root length; **RWRSR**- Relative weight of root and shoot ratio; **PNPP**- Panicle Number per Plant; **PUEstr**-Straw P use efficiency based on P accumulation in straw; **PT**- P translocation; **PTE**- P translocation efficiency; **SPF**- Spikelet Fertility; **TGW**-1000-Grain Weight; **RSL**- Relative Shoot Length; **TNPP**-Total Number of Panicle per Plant; **REP**- Root Elongation under Phosphorus deficiency; **PDW**- Plant dry weight; **RTA**- Relative tillering ability; **RRN**-Relative total root number; **BIOM**- Biomass; **HI**-Harvest index; **PHI**- P harvest index; **RPH**- Relative Plant Height; **RER**-Relative elongation ratio; **RPUC**-Relative P uptaken content; **PCS**- Phosphorus content in shoots; **RRV**-Relative root volume; **GY**-Grain yield; **RSFW**- Relative shoot fresh weight; **RRRS**- Relative root to shoot ratio; **RRFW**- Relative root fresh weight; **GN**- grain number per panicle; **Psi-AAP**- P-deficiency induced activity of acid phosphatase

Kasalath. Of the three QTLs mapped for dry weight, the ones on chromosome 6 and 12 coincided with QTLs for P uptake.

Ni *et al.* (1998), crossed P deficiency tolerant cultivar IR20 to IR55178 -3B-9-3 (sensitive to P-deficiency) and 285 recombinant inbred lines (RILs) were produced by single seed descent method. The RILs were phenotyped for the trait by growing them in P-sufficient (10.0 mg L⁻¹) and P-deficient (0.5 mg L⁻¹) nutrient solution. The RILs and the parents were subjected to AFLP analysis and constructed a map consisting of 217 AFLP markers. Based on the constructed map they identified a major QTL for P-deficiency tolerance, designated as PHO, located on chromosome 12, which is confirmed by RFLP markers RG9 and RG241. They also mapped several minor QTLs on chromosomes 1, 6, and 9.

Feng *et al.* (2000), developed a doubled haploid population from ZYQ8 and JX17 to conduct a QTL mapping for tolerance to low phosphorus. For relative P uptake content they have identified two QTLs which accounts for 20.7% of the total phenotypic variation. One another minor QTL has been found to be associated with relative P utilization efficiency. The study showed that root dry weight is more important trait than others for contributing to p uptake efficiency.

Hu *et al.* (2001), carried out a hydroponic culture experiment with sufficient and deficient phosphorus condition. They have used RILs population developed from cross IR20 and IR55178-3B-9-3 to identify QTLs and epistasis underlying activity of acid phosphatase under phosphorus normal and deficiency condition. Total 4 QTLs identified out of which one for activity of acid phosphatase and three QTLs for P-deficiency induced activity of acid phosphatase (Psi-AAP) on chromosome 1, 6 and 12. Study concluded that Psi-AAP was controlled by a Psi genetic system.

Wissuwa *et al.* (2002), developed a secondary mapping population by backcrossing a near-isogenic line (NIL C443) containing the QTL from the donor parent (Kasalath) to the recurrent parent of low P uptake (Nipponbare) and the QTL mapping showed that close to 80% of the variation between families was due to a single QTL, referred to as *Pup1* (Phosphorus uptake 1). *Pup1* was placed in a 3 cM interval flanked by markers S14025 and S13126, which is within 1 cM of the position identified in the original QTL mapping experiment. Other chromosomal regions and epistatic effects were not significant. Substitution mapping revealed that *Pup1* co-segregated with marker S13126 and that the flanking markers, S14025 and S13752, were outside the

interval containing *Pup1*. They also reported that phenotypic variation between families was due to only one QTL without any additional epistatic interaction.

Shimizu *et al.* (2004), conducted research and observed that root elongation was induced in rice at significant level when grown under phosphorus deficient conditions. They showed the variation in root elongation among 62, varieties screened under two different phosphorus levels. They have identified a single QTL for the elongation response on chromosome 6, with a LOD score of 4.5 in two linkage maps and explained about 20% of total phenotypic variance.

Nguyen and Bui (2006), used a BC₂F₃ developed from cross of low P tolerant Variety AS996 a derivative of *O. rufipogon* and low P sensitive variety OM2395 for mapping QTLs for phosphorus deficiency tolerance. A map consisting of 116 SSR markers was constructed to study associated traits like shoot length, relative root length, relative shoot dry weight and relative dry weight. A major QTL was on chromosome 12 with an additional minor QTLs on chromosome 1, 2, 5 and 9 were identified.

Ping *et al.* (2008), developed a double haploid (DH) population from cross of phosphorus deficiency tolerant japonica rice IRAT109 and phosphorus deficiency sensitive japonica rice Yuefu to do QTL mapping in rice for yield and related traits under phosphorus deficiency. The result obtained from study indicated that seed-setting percentage, panicle number per plant and yield per plant were most easily influenced by phosphorus deficiency than grain weight and grain number. Out of total 17 QTLs detected for plant yield and related trait under phosphorus deficiency, 12 QTLs explained over 10% of phenotypic variation.

Shimizu *et al.* (2008), observed genetic difference in rice for root elongation under phosphorus deficiency and detected a distinct QTL (qREP6) on the long arm of chromosome 6. Further a chromosome segment substitution lines (CSSLs) population used to confirm effect and position of qREP6. Lines with high root elongation under phosphorus deficiency also showed enhanced tillering ability and shoot phosphorus content. They have also found direct interaction between root elongation and root length and root length and tiller number under P deficiency in CSSLs. The fine mapping for qREP6 was done with F₂ population of cross between Nipponbare and CSSL29.

Heuer *et al.* (2009), studied the comparative sequence analyses of the major quantitative trait locus *Phosphorus uptake 1* (*Pup1*) of the Kasalath *Pup1* region against the Nipponbare reference genome. They reported the fine mapping of the *Pup1* locus to

the long arm of chromosome 12 (15.31-15.47 Mb). This study revealed a 278- kbp sequence significantly different from the syntenic regions in Nipponbare (145 kb) and in the *indica* reference genome of 93-11 (742 kbp). Size differences are caused by large insertions or deletions (INDELs), and an exceptionally large number of retrotransposon and transposon-related elements (TEs) present in all three sequences (45%–54%). In their study about 46 kb of the Kasalath sequence did not align with the entire Nipponbare genome, and only three Nipponbare genes (fatty acid alpha-dioxygenase, dirigent protein and aspartic proteinase) were identified as highly conserved in Kasalath. They predicted that two Nipponbare genes (expressed proteins) might have evolved by at least three TE integrations in an ancestor gene that were still present in Kasalath. They also predicted Kasalath genes were novel or unknown genes that are mainly located within INDEL regions may be responsible for low P tolerance.

Tanaka *et al.* (2009), reported the gene expression patterns in two rice genotypes (Nipponbare and NIL6-4 carrying a major QTL for P deficiency tolerance; *Pup1*) grown in soil with and without P fertilizer. They tested the hypotheses that whether tolerance of NIL6-4 is associated with internal P remobilization/ redistribution, enhanced P solubilization and acquisition or root growth modifications that maximize P interception. Genes associated with internal P remobilization/ redistribution and soil P solubilization/ uptake were stress responsive but often more in intolerant Nipponbare. However, genes putatively associated with root cell wall loosening and root hair extension (xylo glucanendo trans glycosylases/hydrolases and NAD (P) H dependent oxido reductase) showed higher expression in roots of tolerant NIL6-4.

Wang *et al.* (2009b), reported the diverse expression patterns of the *OsSPX* genes in different tissues and their responses to P-starvation and they found that five genes, *OsSPX1*, 2, 3, 5 and 6 are responsive to P-starvation in shoots and/or in roots. The sub cellular localization analysis indicated that *OsSPX1* and *OsSPX2* are exclusively located in nucleus, *OsSPX3* in the cytoplasm, and *OsSPX4* is a membrane localization protein. *OsSPX1* regulates *OsSPX2*, 3 and 5 at the transcription level and is positively involved in the responses of the genes to P-starvation. Over expression of *OsSPX3* down regulates *OsSPX5* in shoots under P-sufficiency. They also suggest that *OsSPX1* may be a regulator involved in the transcriptions of *OsSPX2*, 3 and 5. *OsSPX3* plays a role in *OsIPS1*/miR399 mediated long distance regulation on *OsPHO2* indicates that *OsSPX3* is involved in plant tolerance to P-starvation stress.

Li *et al.* (2009), used a set of 271 introgression lines (ILs), to identify QTLs for root traits, biomass and plant height under P-deficiency and P-sufficiency conditions. They used relative MRL (RMRL, the ratio of MRL under P-deficiency to MRL under P-sufficiency condition) and relative RS (RRS) to evaluate P-deficiency tolerance at the seedling stage. They identified a total of 24 additive QTLs and 29 pairs of epistatic QTLs, but only qRN4 was detected in both conditions. QTLs for adaptive traits (RMRL, RRS, RRV, and RRDW) and qRN4 consistently expressed to increase trait stability may contribute to P-deficiency tolerance. They also reported one QTL (qRRS8) showed pleiotropic effects on P-deficiency tolerance and drought tolerance. The promising traits for low P tolerance from this study were relative maximum root length, relative root-shoot ratio, relative root volume and relative root dry weight.

Chin *et al.* (2010), categorized 80 diverse rice accessions into three groups (tolerant Kasalath *Pup1*, intolerant and partial *Pup1*) through the gene based markers that evenly distributed over the 278-kb QTL region of *Pup1* which facilitates targeted introgression of *Pup1* into intolerant varieties by using the gene models predicted in the *Pup1* region in the donor variety Kasalath. Further, they confirmed *Pup1* is conserved among the genotypes adapted to drought-prone environments and broadly absent in rice varieties adapted to irrigated condition. RT-PCR analyses found the up-regulated expression of certain genes from the *Pup1* region such as *OsPupK05* located in the reverse direction within a putative intron of *OsPupK04*, a putative fatty acid oxygenase and *OsPupK20*, a dirigent like gene is restricted to roots with an apparent higher transcript abundance under P-deficient conditions in the *Pup1* NILs and also found that *Pup1* protein kinase gene *OsPupK46-2* is expressed under -P and +P conditions in roots and in shoots. Since this gene is absent from the Nipponbare genome, no expression was detected in Nipponbare and sister NILs without the tolerant *Pup1* locus. Based on the annotation, Gene specific markers were identified, K-20-1 and K-20-2 for *OsPupK20* (Co-dominant) and K-46-2 for *OsPupK46-2* (dominant) that is found in the INDEL region of Kasalath.

Gamuyao *et al.* (2012), performed semi-quantitative Reverse Transcriptase PCR (RT-PCR) and quantitative (q) RT-PCR in contrasting NILs possessing Kasalath *Pup1* locus and confirmed that *OsPupK46-2* was upregulated under P-deficient conditions as sequencing of *Pup1* QTL shows *Pup1*-specific protein kinase gene. The identification of the PSI protein kinase encoding P starvation tolerance 1 (*PSTOL1*) gene conferring P starvation tolerance in rice by enhancing early root elongation, thereby increasing grain

yield in P-deficient soils. Therefore, the *OsPupK46-2* was considered as a candidate gene as its role is most obvious under P-deficient condition and named it as phosphorus starvation tolerance 1 (*PSTOL1*). Over expression of *PSTOL1* in IR-64 (*indica*) and Nipponbare (*japonica*) found to be correlated with enhanced grain yield by more than 60% under low P condition in both varieties. Their analyses showed that *PSTOL1* acts as an enhancer of early root growth, thereby enabling plants to acquire more phosphorus and other nutrients.

Tyagi *et al.* (2012), tested a set of six markers on sixty diverse rice genotypes from North-East and Eastern part of India adapted to acidic soils. They found 21% of the genotypes possessed *Pup1* allele in them, but in irrigate lowland genotypes only 10% of the genotypes were had *Pup1* in them. They identified SahbhagiDhan, Dagaddeshi, Pynthor and Paijiong as potential donors for low soil P tolerance.

Wu *et al.* (2013), proposed the potential application of post-transcriptional regulation of PT1 through *OsPHF1* for P efficiency, which shows that the P uptake, translocation and remobilization are regulated by complex molecular mechanisms through the functions of P transporters (PTs) and other downstream P Starvation Induced (PSI) genes.

Oono *et al.* (2013), isolated RNA from the cultivars grown under Pi starvation and used RNA-Seq profile method to analyze transcriptome analysis among 4 rice cultivars. They have found similarities and difference in comparative analysis of RNA-Seq profile of responsive transcript under Pi starvation condition. A set of new transcript involved in tolerance to Pi starvation has been identified.

Takehisa *et al.* (2013), performed microarray analysis of rice root at 6 and 24 h after phosphorus deficiency treatment. They used cultivar Nipponbare for transcriptome profiling. Analysis was carried out using Rice 4 x 44k Microarray RAP-DB. Among the different unregulated genes under P deficiency condition, a gene encoding multi-copper oxidase found to be major one. Beside this two ferritin genes involved in iron storage in the chloroplast induced by low P deficiency. The additional finding from this study is involvement of ROS signaling in P deficiency response.

Dkhar *et al.* (2014), analyzed the transcriptome data of SahbhagiDhan a popular variety of eastern India and ChakhaoPoreiton a renowned landrace of Manipur in north-eastern India. They selected six genes to validate their functions under stress and control

conditions. The two genes namely, MO5 and MO7 have been identified as showing differential response to low P condition across two distinct rice genotypes.

Mehra *et al.* (2014), reported high quality DNA polymorphisms in low P sensitive genotype, PB1 and tolerant traditional genotype, Dular. Through whole genome resequencing using Illumina NGS platform they identified a total of 5,157,939 sequence variants in PB1 and Dular with reference to Nipponbare genome. They mapped several DNA polymorphisms (non-synonymous and regulatory variants) having potential functional significance to key Phosphate Starvation Responsive (PSR) and root architecture genes in Dular and Kasalath using a compiled list of low P responsive genes. They concluded that the identified variants can serve as a useful source of genetic variability for improving low P tolerance and root architecture of high yielding modern genotypes

Wang *et al.* (2014), conducted a two-year field experiment under low and normal P conditions using a recombinant inbred population of rice derived from cross Zhenshan 97 and Minghui 63 (*indica*). Investigated three yield traits viz., biomass, harvest index, grain yield and eight (P use efficiency) PUE traits like; total P uptake, P harvest index, grain P use efficiency, straw P use efficiency, P use efficiency for biomass and for grain, P translocation and P translocation efficiency. They identified the 36 QTLs and 24 epistatic interactions, among those 26 QTLs and 12 interactions were detected for PUE traits. By comparing their results with similar QTLs in previous studies, three QTLs for PUE traits (qPUP1 and qPUP10 for P uptake, and qPHI6 for PHI) were identified across various genetic backgrounds. They also reported that most QTLs linked to PUE traits were different from those linked to yield traits, suggesting different genetic controls underlying these two traits.

Pariasca-Tanaka *et al.* (2014), tried to carry out improvement of African magarities for P deficiency through marker-assisted introgression of *PSTOL1*. They have determined the *Pup1* haplotype and surveyed the presence or absence of *PSTOL1* and other *Pup1* locus related genes. The new allele for *PSTOL1* in upland NRICAs that was inherited from the *O. glaberrima* parent CG14 has 35 base-pair substitution but maintained fully conserved kinase domain. In current study several allele specific markers developed to use in molecular breeding of African mega varieties for P deficiency.

Aluwihare *et al.* (2015), characterized the K46 DNA marker locus in thirty selected low P tolerance rice cultivars in Sri Lanka. The K46 specific primer-pair was assayed and the PCR products were sequenced. The resulted DNA sequences were aligned with the Kasalath reference sequence for K46 locus. Further cluster analysis of the identified SNPs resulted in four distinct haplotypes in which nine cultivars were grouped with Kasalath like haplotype, two unique haplotypes and the null haplotype. However, they find some with no strong association between the haploype class and the low soil P tolerance score. They described them as potential novel source for low soil P tolerance.

Chao *et al.* (2015), aimed to dissect the genetic basis of low phosphorus tolerance (LPT), 114 BC₂F₄ introgression lines (ILs) were developed from cross of Shuhui527 (recipient parent) and Yetuozai (donor parent). The progenies were tested for 11 quantitative traits under three treatments including normal fertilization in normal soil (as control), normal fertilization in barren soil and low phosphorus stress in barren soil in Langfang, from which 49 QTLs have been detected. Moreover, ILs were investigated at the seedling stage using nutrient solution culture method in greenhouse in Beijing, from which 48 QTLs have been detected. Among all the detected QTLs, eight QTLs were simultaneously identified under low phosphorus stress across two environments.

Yumnam *et al.* (2015), analyzed ten genetically diverse rice genotype for variation at nucleotide loci *PSTOL1* using *PupK20-2* marker, which was reported to be diagnostic for low P tolerance. They have identified two haplotype for *PSTOL1*, lines with high P uptake had Kasalath type haplotype while lines with low P uptake showed mixed haplotype. The discovery of novel SNPs across *PSTOL1* and with respect to the marker, *Pupk20-2* suggest the presence of novel haplotype in genotypes adapted for acidic soil and it can be use in breeding for acidic soil condition.

Gupta *et al.* (2016), used transcriptiome analysis in rice grown under phosphorus deficiency stress. The F₂ population developed form Sahbhagi Dhan and RRF78 used to carry out expression analysis. Variation has been observed for traits like root length, root volume and root surface area. The tolerant lines of F2 population showed higher yield but their root length, root surface area and root volume decreased in P deficient condition which indicate role of PUE (Phosphorus use efficiency) in such genotypes. Study also concluded that increase in root volume or surface area doesn't ensure higher grain yield in P deficiency stress.

Tyagi and Rai (2016), studied root transcriptomes of two indica rice genotypes viz., Sahbhagi Dhan (SD) and Chakhao Poriton (CP) which adapted to acidic soils to understand mechanism of low phosphorus tolerance. Physiological response of SD and CP to low P conditions is very different. While SD shows increase in root length and root biomass, CP maintains its root length and shows decrease in root biomass. Differential up regulation and down regulation of genes observed in both genotype under low P condition. Functional annotation of expressed genes suggests that Sahbhagi Dhan work through serine-threonine kinase *PSTOL1* whereas suberin-mediated cell wall modification seems to be key in Chakhao Poriton. At least six small RNA clusters overlap with transcripts highly expressed under low P, suggesting role of RNA super clusters in nutrient response in plants.

Neelam *et al.* (2017), screened 182 accessions of *O. rufipogon* along with Vandana as a positive control with SSR markers. From the analysis, they inferred that all of the *O. rufipogon* accessions in the study had an insertion of 90 kb region, including *Pup1-K46*, a diagnostic marker for *PSTOL1*. However, they found that it was absent among *O. sativa* cv. PR114, PR121, and PR122. They also sequenced complete *PSTOL1* gene in 67 representative accessions of *O. rufipogon* and Vandana. From comparative sequence analysis, they found 53 mutations (52 SNPs and 1 nonsense mutation) in the *PSTOL1*, coding region, of which 28 were missense mutations and 10 corresponded to changes in the amino acid polarity. These 53 mutations correspond to 17 haplotypes, out of 17 haplotypes, accessions representing eight haplotypes were grown under the phosphorus-deficient conditions in hydroponics for 60 days. Significant differences were observed in the root length and weight among all the genotypes when grown under phosphorus deficiency conditions as compared to the phosphorus sufficient conditions.

Zhang *et al.* (2017), analyzed RILs from cross XieqingzaoB (XB) and Zhonghui9308 for identifying genomic regions contributing for tolerance to Pi deficiency. A Number of traits related to tolerance to Pi deficiency were evaluated for QTL analysis during tillering and heading stages. Most of the traits were correlated to each other. Total 21 additive QTLs were detected with ranging phenotypic variation between 10-49%, tending to cluster on chromosome 4, 6, 10 and 11. Among all QTLs, qTPDE4, showed most stable tolerance against P deficiency.

Luo *et al.* (2017), used backcross recombinant inbred lines derived from inter-specific cross (*O. sativa* L. X *O. rufipogon* Giff) for QTL identification of P-deficiency

tolerance. Out of total twenty-three QTLs, eight QTLs showed high contribution to phenotypic variation. This study represented a first step in identification of low P stress tolerance genes from *O. rufipogon*.

Giri *et al.* (2018), showed involvement of *OsAUX1* gene in low external phosphate tolerance by facilitating root hair elongation. Disrupting the *OsAUX1* auxin influx transporter gene causes change in root angle that affects rice P acquisition efficiency. Thus auxin and *OsAUX1* play key roles in promoting root foraging for P in rice.

Anis *et al.* (2018), investigated a population of 76, chromosomal segment substitution lines (CSSLs) for identification of QTL underlying related traits for phosphorus deficiency tolerance. They have detected three QTLs under low phosphorus, one under normal phosphorus and three under their relative ratio on the rice chromosome 3, 5, 6, 8 and 10 with phenotypic variation ranging from 10.82 to 18.46%.

MATERIAL AND METHODS

Chapter III

MATERIAL AND METHODS

The present study entitled “Molecular mapping of genetic loci associated with tolerance to low soil phosphorus in rice (*Oryza sativa* L.)” was conducted at ICAR-Indian Institute of Rice Research (ICAR-IIRR, formerly known as Directorate of Rice Research) and Institute of Biotechnology (IBT), PJTSAU, Rajendranagar, Hyderabad, India. The study was carried out with the following objectives;

1. Characterization of *Pup1* locus in a set of landraces of rice to identify novel donor for low soil P tolerance using a set of locus specific molecular markers
2. Development of mapping populations for low soil P and screening for low P tolerance. Mapping of genomic regions associated with low soil P tolerance and identification of closely linked QTLs/genes
3. *In-silico* identification of putative candidate genes associated with low soil P tolerance using mapping data

3.1 Screening of germplasm to identify low phosphorus tolerance lines in low phosphorus soils

3.1.1 Plant material:

Germplasm lines collected (Table.3.1) from Dr. L. V. Subbarao, Principal Scientist, Crop improvement Section, Plant Breeding ICAR-IIRR, Rajendranagar, Hyderabad, were used for screening for low soil P tolerance during Kharif 2017. Swarna, (low soil P tolerant) and Improved Samba Mahsuri (ISM) (Low soil P susceptible) were used as positive and negative checks respectively during the screening.

Table 3.1: List of germplasm lines used for checking the allelic status among the germplasm lines with respect to *Pup1*

Sl no	Germplasm lines		Sl no	Germplasm lines
1	IC216610		29	IC217027
2	IC216682		30	IC216997
3	IC216686		31	IC217026
4	IC216736		32	IC217052
5	IC216739		33	IC217073
6	IC216701		34	IC217075
7	IC216731		35	IC217082
8	IC216767		36	IC217077
9	IC216819		37	IC217107
10	IC216823		38	IC217120
11	IC216798		39	IC217124
12	IC216820		40	IC217275
13	IC216829		41	IC217293
14	IC216806		42	IC217339
15	IC216810		43	IC217345
16	IC216831		44	IC217347
17	IC216840		45	IC217357
18	IC216938		46	IC217366
19	IC16901		47	IC217515
20	IC216903		48	IC217519
21	IC16909		49	IC217566
22	IC216934		50	IC217567
23	IC216948		51	IC217609
24	IC216956		52	IC217646
25	IC216957		53	IC217678
26	IC216942		54	IC217684
27	IC216976		55	IC217680
28	IC217016		56	IC217688

3.1.2 Methodology:

3.1.2.1 Phenotypic screening

3.1.2.1.1 Phenotypic screening under low soil P condition

Phenotypic screening under low soil P was done by planting the germplasm lines in low soil P plot of ICAR-IIRR. All the recommended agronomic practices except P fertilizers application were followed to ensure good crop. The available soil P and pH of the soil were measured before taking up of the planting in low P plot (Table.3.2). The observations on following parameter were recorded during the crop growth period under low P soil and average data considered from three plants;

1. Days to 50% flowering (Nos.)

DFF recorded as the number of days taken by each genotype from sowing to opening of the first flower in 50% of the plants.

2. Plant height (cm)

The height of the three randomly selected plants was measured from base of the shoot to the tip of the main stem. The average height was worked out and expressed in centimeters.

3. Number of productive tillers per plant (Nos.)

The total number of productive tillers per plant in three randomly selected plants per germplasm lines were recorded at harvest. The mean number of tillers per plant was worked out and expressed in number of tillers per plant.

4. Flag leaf length (cm)

Flag leaf length was measured in centimetre from the base of flag leaf to its tip, during flowering and an average of three plants considered for final reading.

5. Flag leaf width (cm)

Flag leaf width was measured in centimetre at broader place of leaf blade in the centre of the leaf, during flowering.

6. Panicle length (cm)

Panicle length was measured as the distance from the panicle node to the tip of the panicle and expressed in centimeters and mean value was calculated.

7. Shoot length (cm)

Average length of shoot was taken by uprooting the plants and measured from base of the shoot to tip of the longest leaf.

8. Root length (cm)

Three plants per germplasm lines were randomly selected and used for measuring root traits after carefully uprooting the plants. The maximum root length (MRL) was measured from collar region to the tip of longest root with the help of a meter scale and average root length was computed and expressed in centimeters.

9. Grain yield per plant (g)

In each entry, total number of grains from three plants were harvested at maturity, threshed, cleaned and dried (15-18 percent moisture content) and the weight

was recorded. An average value of these three plant yield was considered as grain yield per plant.

10. Dry shoot weight and (g)

Shoots that were previously used to measure shoot length were taken for their dry weight determination. Samples were sun dried initially and then kept in oven at 80°C for a period of three days and oven dried samples were weighed using electronic balance to determine their dry shoot weights.

11. Root volume (ml)

Root volume was measured by using water displacement method. Plants were uprooted carefully without causing any damage to root system, soil was washed out and excess water that remained on the surface of the roots was removed by blotting with absorbent paper. Then the roots were placed in measuring cylinder containing water, rise in the level of water in the measuring cylinder was calculated by subtracting final volume (level of water after placing the root in the measuring cylinder) from the initial volume (level of water before placing the root in the measuring cylinder) and expressed it as root volume in ml.

12. Total biomass (g)

Total biomass was taken at time of complete maturity. After maturity plants were cut at the base, dried completely and weights were taken using portable filed electronic balance.

Table 3.2: Status of soil nutrients and pH in low P plot at ICAR-IIRR, Hyderabad during *Kharif*, 2017

SI. no	Soil attributes	Status	Remarks
1	Soil Nutrient		
	Available Nitrogen kg ha ⁻¹	121.5	Low
	Available Phosphorus kg ha ⁻¹	1.41	Very Low
	Available Potassium kg ha ⁻¹	596	High
2	Soil pH	6.8	Neutral

3.1.2.1.2 Phenotypic screening under normal P condition

The same set of germplasm lines planted in low soil P plot, were planted in normal field (available P > 24 kg ha⁻¹), applied with recommended dose of Nitrogen (N), Phosphorus (P) and Potassium (K) with resistance and susceptible checks. All the recommended agronomic practices were followed to ensure good crop and following

observations were recorded as average data from three plants calculated as explained in section **3.1.2.1.1**.

1. Days to 50% flowering (Nos)
2. Plant height (cm)
3. Number of productive tillers per plant (Nos)
4. Flag leaf length (cm)
5. Flag leaf width (cm)
6. Thousand grains weight (g)
7. Grain yield per plant (g)

3.1.2.2 Gene profiling Study:

Genotypic screening was done by employing *Pup1* associated markers (Table. 3.3) reported by Chin *et al.* (2011), Tanaka *et al.* (2014). Standard protocol for DNA isolation and PCR were followed. Amplified products were resolved using agarose gel electrophoresis. Details of the protocols followed for DNA isolation, PCR and gel electrophoresis was summarized as follows;

3.1.2.2.1 DNA isolation

Cetyl Tri Methyl Ammonium Bromide (CTAB) method of Murray and Thompson (1980) used to isolate DNA from leaf. DNA isolation buffer was prepared by using the following stock solutions;

Preparation of Stock Solutions

1 M Tris HCl: 30.275 g of 1 M Trizma base (Trishydroxy methyl amino methane- mol. wt. 121.1) was dissolved in about 180 ml distilled water and then pH was adjusted to 8.0 using 1 N HCl and finally the volume made up to 250 ml. This stock solution was autoclaved and stored at room temperature.

0.5 M EDTA: 46.53 g of Ethyl diamine tetra acetic acid (EDTA-Mol. Wt. 372.24) was dissolved in the 180 ml of distilled water then pH of the solution was adjusted to 8.0 with 1 N NaOH and volume was made to 250 ml. This stock solution was autoclaved and stored at room temperature.

2.5 M NaCl: 36.5 g of NaCl (Mol. Wt. 58.44g) was dissolved in 250 ml of distilled water. The solution was autoclaved and stored at room temperature

DNA Isolation protocol

1. Two to three large leaf pieces (weighing 2-3 g) were collected from the plants growing in field, in a labeled plastic cover, kept in freezer pack. After bringing the plastic cover to lab, they were immediately stored at -20⁰C.
2. 2 cm leaf pieces were cut into small bits in a sterile porcelain spot plate and 350 µl of C-TAB buffer was added. The leaf bits were ground into fine using a sterile glass rod, and added another 350 µl of C-TAB buffer.
3. The contents from the spot plate transferred to labeled 1.5 ml micro centrifuge tubes using a 1000 µl capacity micropipette with micropipette tips cut at their tip portion.
4. Equal volume of Chloroform was added to each micro centrifuge tube. The contents were mixed well by inversion for about 10 min and centrifuged at 13,000 rpm for about 15 min at room temperature.
5. After centrifugation, the supernatant was aliquoted from the micro centrifuge tube into freshly labeled sterile 1.5 ml micro centrifuge tubes. To this 1/8 volume of 3M sodium acetate (pH 5.2) and equal volume (500-600µl) of chilled Isopropyl alcohol was added and kept in freezer for 15 min. The contents were mixed gently and centrifuged at 13,000 rpm for 10 min at room temperature.
6. The supernatant was drained gently and about 100µl of 70% ethanol was added to the pellet. The tube was tapped gently so that the pellet was disturbed. Centrifugation was done at 13,000 rpm for 5 min at room temperature. After this the supernatant was drained out and the pellet was left out for air drying overnight at room temperature with the tube cap open.
7. After complete drying of pellet, 50-100 µl of sterile TE buffer was added to dissolve the pellet. The DNA was stored at 4⁰C for further analysis.

3.1.2.2.2 Analysis of DNA quantity and quality

Quality analysis of genomic DNA by agarose gel electrophoresis:

Open ends of molded Acrylic gel casting plate (Thermo Scientific. USA) were sealed with cello tape and the comb was placed properly on casting plate, plate kept on a perfectly horizontal platform. 0.8 % (0.8 g/100 ml) agarose was added to 1X TBE (for 10X TBE 108 g of Tris base, 55 g of Boric acid and 9.8 g of EDTA) boiled until the agarose was dissolved and then allowed to cool. To stain the DNA, Ethidium bromide (10 mg/ml of distilled water) was added when temperature reached 55-60⁰C and poured

into the gel mould and allowed to solidify. The comb and cello tape were removed carefully after solidification. The casted gel was placed in the electrophoresis unit contained 1X TBE, seen that the gel was properly submerged.

Loading DNA Samples:

Four microliter of DNA sample was then mixed with 2 μ l of 1X bromophenol blue loading dye (0.0025% bromophenol blue in 40% sucrose), mixed thoroughly by using pipette up and down several times, then loaded in the wells and electrodes were connected to the power pack (CBS Scientific, USA). The gel was run at 8 V/cm for 1-1.5 hours. The bands were visualized under UV illuminator and documented using a gel documentation system (Model Alpha Imager 1200, Alpha Innotech corp, US).

Dilution of DNA:

Genomic DNA quality and quantity was checked through NanoDrop method as well as by agarose gel electrophoresis. The final concentration of all the DNA samples was adjusted to 50 ng/ μ l. The diluted DNA was subsequently used for PCR amplification.

3.1.2.2.3 PCR conditions for analysis.

PCR analysis was done to identify the presence or absence of *Pup1* associated marker loci, by using forward and reverse microsatellite primers, Reactions were performed in a final volume of 20 μ l. 3 μ l of diluted total genomic DNA, 2.0 μ l of 10X PCR buffer (10mM Tris-HCl pH 9.0, 50 mM KCl, 1.5 mM MgCl₂ and 0.01% gelatin), 1 μ l of dNTPs solution (2.5mM), 1 μ l of each of forward and reverse primers (5 pM), 1 μ l of 1.0 U *Taq* DNA polymerase (Banglore genei Private Limited, Banglore) and 11 μ l of sterilized double distilled water. Amplification was carried out using programmable Thermal cycler (G- Strom and Eppendorf). The PCR protocol adopted for amplifying the *Pup1* associated marker loci are summarized below;

94°C: 5 minutes (initial denaturation)

94°C: 30 seconds (denaturation)

55-58°C: 30 seconds (annealing)

} Repeated for 30-35 cycles

72°C: 1 minute (extension)

72°C: 7 minutes (final extension)

3.1.2.2.4 Resolution of PCR fragments and scoring

Agarose gel preparation:

Preparation of agarose gel has carried out as explained in section **3.1.2.2.2**

Loading DNA Samples and electrophoresis:

10µl of DNA sample was then mixed with 3 µl of 1X bromophenol blue loading dye (0.0025% bromophenol blue in 40% sucrose), mixed thoroughly by using pipette up and down several times, then loaded in the wells and electrodes were connected to the power pack (CBS Scientific, USA). The gel was run at 8 V/cm for 1-1.5 hours. The bands were visualized under UV illuminator and documented using a gel documentation system (Model Alpha Imager 1200, Alpha Innotech corp, US).

3.2 Development of mapping population for low soil P tolerance and mapping of genomic regions associated with low soil P tolerance to identify closely linked genes/QTLs

3.2.1 Plant materials:

3.2.1.1 Donor parent

Wazuhophek is a landrace from north-east India and it displayed excellent tolerance to low soil P while undertaking phenotypic screening at low soil phosphorus plot (Olsen P: 2.04 ppm P) at ICAR-IIRR. However, Wazuhophek is devoid of the well known low P tolerance QTL, *Pup1* (based on analysis with functional markers) and hence may harbor novel genes/QTLs associated with tolerance (Swamy *et al.*, 2019)

3.2.1.2 Recipient parent: Improved Samba Mahsuri (ISM)

Improved Samba Mahsuri is an essentially derived variety (EDV) of Samba Mahsuri (BPT5204), developed through marker-assisted pyramiding of three bacterial

blight resistance genes viz., *Xa21*, *xa13* and *xa5* in the genetic background of Samba Mahsuri to confer broad spectrum resistance to bacterial blight (Sundaram *et al.*, 2008). Improved Samba Mahsuri (ISM) is steadily replacing Samba Mahsuri variety in many parts of south India and it retains the high yield, medium slender grain type and excellent cooking quality of Samba Mahsuri. ISM was observed to be very sensitive to low soil P, when screened in the low P plot of ICAR-IIRR (Anila *et al.*, 2017).

3.2.2 Crossing program for developing mapping population

To develop a mapping population for identification of genes/QTLs, associated with low soil P tolerance in Wazuhophek, a cross was made between Wazuhophek and Improved Samba Mahsuri during *Rabi*, 2014-15. Single seed descent method was adopted for advancing the mapping population and development of recombinant inbred lines (RILs).

3.2.2.1 Development of mapping population

A mapping population of 112 lines was developed at ICAR-IIRR by advancing the F_2 plants derived from the cross Wazuhophek /ISM to $F_{6:7}$ generations by adopting single seed descent (SSD) method. Wazuhophek was used as the donor parent while ISM was a recipient parent. To avoid self-fertilization (as rice is a self-pollinated crop), the female parent was emasculated; a middle portion of the panicle was selected for emasculation after removing the upper and lower portion. Emasculation was carried out during the evening hours by cutting the spikelet with the help of small scissors (clipping method of emasculation) so that anthers could be easily visible and removed carefully with the help of fine-pointed forceps without damaging the female floral parts. The emasculated panicles were then covered with glassine bags to avoid pollination from unwanted source. The panicles from Wazuhophek were collected in the morning, during peak anthesis period i.e., between 9.00 AM to 11.00 AM were used for pollination, pollinated panicles were covered with glassine bags to avoid cross contamination from external pollen source. Twenty five days after pollination, F_1 seeds were harvested from individual plants, dried and preserved for further use.

3.2.3. Survey of parental polymorphism between Wazuhophek and ISM using rice microsatellite markers

A set of hyper-variable rice microsatellite markers were used to check polymorphism between donor parent, Wazuhophek and susceptible parent of mapping

population, ISM. Among a total of 587 SSR markers spread across the 12 chromosomes, which were tested, 110 polymorphic SSR markers were identified out of which 78 SSR markers and used for analysis of the mapping population. The chromosome location and base sequence of forward and reverse primers of SSR markers is given in Appendice C. The sequence information of these primers is also available at <http://www.gramene.org>. For the parental polymorphic survey, DNA was isolated from the leaf samples, using Cetyl Tri Methyl Ammonium Bromide (CTAB) method of Murray and Thompson (1980). Nanodrop and Agarose gel electrophoresis methods used to check quantity and quality of the DNA. The PCR and gel electrophoresis protocols mentioned in the **chapter 3.1.2.2.3** and **chapter 3.1.2.2.4** respectively were followed and ethidium bromide stained 3% to 3.5 % agarose gels were used to resolve the PCR products.

3.2.4 Genotyping of mapping population

3.2.4.1 Collection and preservation of leaf material

Single plant was elected from each 112 mapping population lines (RILs) and parents and leaves were collected from selected plants from the field. The leaves were collected in plastic bags and immediately kept in the ice box and stored at -20⁰ C for further use.

3.2.4.2 Genomic DNA isolation

DNA was isolated by following the protocol of Murray and Thompson (1980) with modifications as mentioned in section 3.1.2.2.1.

3.2.4.3 Analysis of quality and quantity of DNA isolated

The quality and quantity of isolated DNA were checked through Ethidium bromide stained 0.8 % Agarose gel electrophoresis (AGE) as mentioned in section 3.1.2.2.2.

3.2.4.4 Scoring of gels for mapping population

Genotyping data was recorded using symbols like A (Wazuhophek type allele), B (ISM type allele), H (Heterozygote type allele) in excel sheet.

3.2.5 Evaluation of mapping population for low P tolerance and for agro-morphological traits

Mapping population (RILs) developed from cross Wazuhophek and ISM were evaluated in low P soil plot of ICAR-IIRR during Kharif 2017. Available soil P and soil pH of low P plot was measured before taking up the planting. Seedlings were raised by using wet bed nursery. Mapping population (RILs) were planted in low P plot of ICAR-IIRR along with the donor and recipient parents and proper check and were also planted in normal plot for further advancement and evaluation for agro-morphological traits.

In low P soil plot, plants were applied with recommended dose of nitrogen, potassium and zinc with no application of Phosphorus. All the recommended agronomic practices were followed. In the normal plot plants were applied with recommended doses of NPK and all the recommended agronomic practices were followed. The following observations were recorded from both low soil P plot and data for selected traits from normal soil P plot and it represented as average data from three plants as explained below;

1. Days to 50% flowering (DFF)

DFF recorded as the number of days taken by each genotype from sowing to opening of the first flower in 50% of the plants.

2. Plant height (cm)

The height of the three randomly selected plants was measured from base of the shoot to the tip of the main stem. The average height was worked out and expressed in centimeters.

3. Number of productive tillers per plant

The total number of tillers per plant in three randomly selected plants per accession was recorded at harvest. The mean number of tillers per plant was worked out and expressed in number of tillers per plant.

4. Flag leaf length (cm)

Flag leaf length was measured in centimeters from the base of flag leaf to its tip, during flowering and an average of three plants considered for final reading.

5. Panicle length (cm)

Panicle length was measured as the distance from the panicle node to the tip of the panicle and expressed in centimeters and mean value was calculated.

6. Shoot length (cm)

Average length of shoot was taken by uprooting the plants and measured from base of the shoot to tip of the longest leaf.

7. Root length (cm)

Three plants per accessions were randomly selected and used for measuring root traits after carefully uprooting the plants. The maximum root length (MRL) was measured from collar region to the tip of longest root with the help of a meter scale and average root length was computed and expressed in centimeters.

8. Grain yield per plant (g)

In each entry, total number of grains from three plants were harvested at maturity, threshed, cleaned and dried (15-18 percent moisture content) and the weight was recorded. An average value of these three plant yield was considered as grain yield per plant.

9. Dry shoot weight and (g)

Shoots that were previously used to measure shoot length were taken for their dry weight determination. Samples were sun dried initially and then kept in oven at 80°C for a period of 3 days and oven dried samples were weighed using electronic balance to determine their dry shoot weights.

10. Dry root weight (g)

Roots that were previously used to measure root length were taken for their dry weight determination. Samples were sundried initially and then kept in oven at 80°C for a period of 3 days. The oven dried samples were weighed using electronic balance to determine their dry root weights

11. Root volume (ml)

Root volume was measured by using water displacement method. Plants were uprooted carefully without causing any damage to root system, soil was washed out and excess water that remained on the surface of the roots was removed by blotting with absorbent paper. Then the roots were placed in measuring cylinder containing water, rise in the level of water in the measuring cylinder was calculated by subtracting final volume (level of water after placing the root in the measuring cylinder) from the initial volume (level of water before placing the root in the measuring cylinder) and expressed it as root volume in ml.

12. Thousand grains weight (g)

Seeds harvested from each genotype were dried, counted thousand numbers in electronic grain counter and weighed using electronic balance to measure thousand grains weight.

13. Root to shoot ratio by length

Average shoot weight (gm) and average root weight (gm) was taken and root to shoot ratio was calculated on weight basis in percentage (%) as per the below formula.

$$\text{Root to shoot ratio (\%)} = \frac{\text{Average root weight (gm)}}{\text{Average shoot weight (gm)}} \times 100$$

14. Total biomass (g)

Total biomass was taken at time of complete maturity. After maturity plants were cut at the base, dried completely and weights were taken using portable field electronic balance.

15. P content of grains (mgg⁻¹)

One gram of de-husked grain was digested with a 9:4 mixture of nitric acid and perchloric acid on a hot plate. The clear digested residue was cooled, diluted to 100 ml distilled water and filtered to remove insoluble silica. The digested extract was used to estimate the phosphorus content by Vanado–molybdo phosphoric yellow color method (Piper, 1966) using Spectrophotometer (Elico SL – 177) at 420 nm and P content was expressed as percent.

3.3 Mapping of genomic regions associated with low soil P tolerance to identify closely linked genes/QTLs

3.3.1 Linkage map construction

Linkage analysis and map construction were performed using a total of 78 polymorphic SSR markers and the program IciMapping version 4.0 and Win QTL cartographer 2.5, the map distances were converted into cM, using the Kosambi function (Kosambi, 1944). With QTL IciMapping version 4.0, three steps namely grouping, ordering, and rippling were followed in order, after which using outputting command, the complete linkage map was obtained. Linkage groups were obtained using two-point analysis with a threshold LOD score of 2.5. For QTL analysis by Win QTL cartographer 2.5, linkage map construction done using MAPMAKER.

3.3.2 QTL mapping

QTL analysis was performed with QTL IciMapping version 4.0 using ICIM (Inclusive composite interval mapping) for molecular mapping of the low soil P tolerance associated loci. LOD of 2.5, was set as a threshold for identification of significant QTL. The genotype data for the polymorphic markers were used together with the phenotypic data with respect to tolerance/susceptible RILs in the analysis. The results obtained were rechecked with Win QTL Cartographer 2.5 (Wang *et al.*, 2005a) version 2.5 using CIM (composite interval mapping). Based on permutation tests performed 1,000 times at $\alpha = 0.05$ for experiment-wise Type I error and referring to the empirical threshold values widely used for QTL mapping, LOD value of 2.5 was set as the threshold for identification of QTL. Nomenclature for QTL followed as described by McCouch *et al.* (1997). Two or three letters abbreviated from the trait name followed by chromosome number of rice where the QTL is found and a terminal suffix, separated by a period, gives a unique identifier to distinguish multiple QTL on a single chromosome. QTL whose LOD is more than 2.5 and contributing more than 10% of the total phenotypic variance with large additive effect on the trait were considered as major effect QTL.

3.3.3 *In-silico* identification of putative candidate genes associated with low soil P tolerance using mapping data

In order to navigate for the presence of any candidate genes present in the chromosomal regions associated with low soil P tolerance, information from QTLs mapped in current study were used for *in-silico* analysis with rice database; RAP-DB, QTARO database and Rice genome annotation project

(<http://rapdb.dna.affrc.go.jp/>, <http://qtaro.abr.affrc.go.jp/>, http://rice.plantbiology.msu.edu/analyses_search_locus.shtml). Gene list for chromosome 1 (1 to 10 Mb), chromosome 7 (4 to 16 Mb and 23 to 27 Mb) and chromosome 8 (0 to 5 Mb) regions was downloaded from RAP-DB. Obtained genes were grouped according to their annotation and their role in low soil P tolerance.

3.4 Statistical Analyses for morphological characters for the germplasm lines and RILs

Three plants were selected randomly and data on individual mean was subjected to statistical analysis. The data was analyzed using SPSS v20 software programme. For the analysis of the data the following statistical methods were employed.

3.4.1 Mean: Mean value of each character was determined by dividing the sum of the observed value with the corresponding number of observations.

3.4.2 Standard error: The standard error is a measure of the precision of sample mean. To know how widely they are scattered some measurements like standard deviation is used and to indicate the uncertainty around the estimate of mean measurement, the standard error of the mean is quoted.

3.4.3 Range: Lowest and highest values for each character were recorded and expressed as range.

3.4.4 Co-efficient of variability

Both genotypic and phenotypic coefficients were computed for each character as per the method suggested by Burton and Davane (1953).

$$\text{Genotypic coefficient of variability (GCV)} = \frac{\sigma_g}{\bar{X}} \times 100$$

$$\text{Phenotypic coefficient of variability (PCV)} = \frac{\sigma_p}{\bar{X}} \times 100$$

Where,

σ_g = Genotypic standard deviation,

σ_p = Phenotypic standard deviation

$\bar{X}$ = General mean of character

GCV and PCV values were categorized as low, moderate and high values as suggested by Sivasubramanian and Madhavamenon (1973), as follows.

1-10 per cent	: Low
10-20 per cent	: Moderate
20 per cent and above	: High

3.4.5 Heritability (h^2)

Heritability in broad sense was computed as the ratio of the total genotypic and phenotypic variance as expressed in percentage as given by Allard (1960).

$$\text{Heritability } (h^2) = \frac{\sigma^2_g}{\sigma^2_p} \times 100$$

Where,

σ^2_g = Genotypic variance

σ^2_p = Phenotypic variance

3.4.6 Genetic advance (GA)

Genetic advance was estimated by using the formula given by Johnson *et al.* (1955).

$$GA = h^2 k \sigma_p$$

Where,

h^2 = Heritability in broad sense

k = Selection differential which is equal to 2.06 at 5 per cent intensity of selection

p = Phenotypic standard deviation

3.4.7 Genetic advance as percent of mean (GAM)

$$GAM = \frac{GA}{\bar{X}} \times 100$$

Where,

GA = Genetic advance

$\bar{X}$ = General mean of the character

It is as follows,

0-10 per cent : Low

10-20 per cent : Moderate

20 per cent and above: High

3.4.8 Correlation analysis

The correlation coefficients were worked out to determine the degree of association of a character with yield and also among the yield components. Genotypic and phenotypic correlations were computed by using the formula given by Weber and Moorti (1952).

$$r_p = \frac{\text{Cov}(xy)_p}{\sigma_{px} \times \sigma_{py}} \times 100$$

$$r_g = \frac{\text{Cov}(xy)_g}{\sigma_{gx} \times \sigma_{gy}} \times 100$$

Where,

r = Correlation coefficient

Cov (xy) = Covariance between the characters 'x' and 'y'

px and py = Phenotypic variance of the character 'x' and 'y' respectively

gx and gy = Genotypic variance of the character 'x' and 'y' respectively

Correlation coefficients were compared against 'r' values given in Fisher and Yates (1963) table at (n-2) degrees of freedom at the probability levels of 0.05 and 0.01 to test their significance.

3.4.9 Cluster Analysis

The cluster analysis using Euclidean distance, with Unweighted Pair Group Method using Arithmetic means (UPGMA) based on low P soil tolerance indices, yield under normal condition and yield under low soil P condition (Stress) was done in DARwin 6 (Perrier and Jacquemoud-Collet, 2006).

3.4.10 Calculation of stress Indices

To evaluate response of genotypes to low P stress, some stress indices based on a mathematical relation between stress- and optimum conditions has been worked out as follows;

Sl no.	Index Name	Formula	Reference
1	Stress Tolerance Index (STI)	$STI = \frac{Y_P Y_S}{(\bar{Y}_P)^2}$	Fernandez, 1992
2	Tolerance Index	$TOL = Y_P - Y_S$	Rosielle and Hamblin, 1981
3	Stress Susceptibility Index (SSI)	$SSI = \frac{1 - Y_S/Y_P}{1 - \bar{Y}_S/\bar{Y}_P}$	Fisher and Maurer, 1978
4	Yield Stability Index (YSI)	$YSI = \frac{Y_S}{Y_P}$	Bousslama and Schapaugh, 1984
5	Yield Reduction Ratio (YR)	$YR = 1 - \left(\frac{Y_S}{Y_P} \right)$	Golestani-Araghi and Assad, 1998
6	Yield Index (YI)	$YI = \frac{Y_S}{\bar{Y}_S}$	Gavuzzi <i>et al.</i> , 1997
7	Percent yield reduction	$YR = \frac{Y_P - Y_S}{Y_P} \times 100$	Yaseen and Malhi, 2009

Where,

YS = Yield under low soil P stress condition

YP = Yield under normal/Sufficient P condition

$\bar{Y}_S$ = Mean yield under low soil P stress condition

$\bar{Y}_P$ = Mean yield under normal/Sufficient P condition

RESULTS AND DISCUSSION

Chapter IV

RESULTS AND DISCUSSION

4.1 Screening of germplasm lines to identify low phosphorus (P) tolerance lines under low soil P condition

4.1.1 Screening of germplasm lines to identify low soil P tolerance lines in low soil P condition

4.1.1.1 Performance of rice germplasm lines under low soil P and normal soil P conditions

4.1.1.2 Estimation of phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV), heritability and genetic advance under low P condition

4.1.1.3 Assessment of stress tolerance indices among the rice germplasm based on their performance in low soil P and normal P soils

4.1.2 Genotypic screening of germplasm lines using *Pup1* specific molecular markers

4.1.3 Identification of novel source for low P tolerance

4.1.1 Screening of germplasm to identify low soil P tolerance lines in low soil P condition

In order to identify the novel source for low soil P tolerance, a total of 56 germplasm lines collected were screened in low soil P plot at ICAR-IIRR (available P, <2 kg ha⁻¹). Before planting, the soil of low soil P plot was analyzed to know the nutrient status. The available N was low, available P was very low, available K was high and p^H was neutral (Muhr *et al.*, 1965; Thakur *et al.*, 2014). The field was applied with recommended dose of N, K and Zn at the time of planting and N in splits based on RKMP ‘fertimeter’ recommendations (www.rkmp.co.in)

4.1.1.1 Performance of rice germplasm lines under low soil P and normal soil P conditions

Under low P condition, eleven traits were measured among the 56, germplasm lines of rice along with positive check Swarna (low soil P tolerant) and negative check Improved samba mahsuri (low soil P susceptible). Performance of 56, germplasm lines

Table 4.1 : Performance of rice germplasm lines in low soil P condition at ICAR-IIRR, Hyderabad, during *Kharif*, 2017

Sl no	Germplasm lines	Days to 50% flowering (Days)	Plant height (cm)	Number of tillers(nos.)	Flag leaf length (cm)	Flag leaf width (cm)	Panicle length (cm)	Root length (cm)	Root volume (ml)	Shoot length (g)	Dry shoot weight (g)	Biomass (g)	Grain yield per plant (g)
1	Swarna	125.00	65.00	8.00	20.50	1.17	18.70	25.40	27.60	62.90	14.95	17.65	9.00
2	ISM	122.00	51.00	2.00	16.30	0.70	11.00	22.00	12.00	48.00	2.00	5.50	2.50
3	IC216610	116.00	103.70	8.30	22.50	1.00	22.10	28.50	15.00	95.50	4.40	7.50	3.10
4	IC216682	117.00	95.00	2.70	25.70	0.70	19.70	28.00	25.00	96.50	3.10	6.50	3.40
5	IC216686	117.00	90.70	7.00	21.30	0.90	19.30	25.00	60.00	80.00	7.00	14.50	7.50
6	IC216736	119.00	95.00	5.30	21.70	0.90	18.30	29.00	40.00	98.00	1.10	5.10	4.00
7	IC216739	117.00	71.70	4.00	17.00	0.90	20.00	24.50	30.00	83.00	2.20	5.20	3.00
8	IC216701	120.00	64.20	4.00	13.70	0.60	13.50	22.50	5.00	76.00	1.60	1.90	0.30
9	IC216731	113.00	97.70	10.00	20.80	0.80	18.50	27.50	45.00	87.00	16.60	23.70	7.10
10	IC216767	104.00	91.00	4.00	24.00	0.70	16.50	26.00	25.00	80.00	4.30	6.30	1.90
11	IC216819	101.00	80.00	5.70	18.30	0.60	16.30	30.50	45.00	93.50	1.80	1.80	0.00
12	IC216823	104.00	70.80	8.70	23.50	0.60	15.20	21.50	25.00	74.50	6.00	8.80	2.80
13	IC216798	111.00	70.00	6.70	28.20	0.70	14.50	21.50	18.00	66.50	0.00	3.70	3.70
14	IC216820	119.00	94.00	5.00	18.70	0.70	19.30	22.00	40.00	99.00	1.70	5.00	3.30
15	IC216829	100.00	67.00	3.70	23.30	0.60	14.80	21.00	18.00	65.00	0.80	1.60	0.80

16	IC216806	123.00	90.00	7.30	20.30	0.80	17.70	27.00	38.00	98.50	6.30	11.80	5.40
17	IC216810	121.00	71.70	4.00	17.80	0.70	15.00	20.50	18.00	97.50	3.50	6.20	2.70
18	IC216831	121.00	80.00	5.70	18.80	0.80	15.70	23.50	33.00	76.50	9.50	19.10	9.60
19	IC216840	114.00	86.00	6.00	24.20	0.50	17.00	22.50	45.00	86.50	0.80	1.80	1.00
20	IC216938	114.00	105.30	5.30	18.70	0.80	21.00	20.50	23.00	104.00	12.30	18.90	6.60
21	IC16901	114.00	111.50	4.00	29.50	1.00	23.50	30.00	23.00	125.00	5.30	10.50	5.20
22	IC216903	116.00	87.70	9.30	25.30	0.70	21.30	25.50	50.00	91.50	2.50	7.60	5.10
23	IC16909	111.00	85.70	3.00	20.70	0.80	15.30	23.50	45.00	98.00	4.60	7.50	2.90
24	IC216934	109.00	91.00	6.30	18.70	0.90	18.30	26.50	25.00	104.00	13.70	19.20	5.50
25	IC216948	113.00	95.00	4.70	20.00	0.90	18.30	21.00	18.00	96.50	4.40	6.70	2.30
26	IC216956	113.00	86.70	7.00	23.30	0.60	21.30	28.00	30.00	89.00	7.50	10.00	2.50
27	IC216957	111.00	86.00	5.30	26.30	0.60	20.30	26.50	30.00	101.00	7.70	10.50	2.80
28	IC216942	118.00	78.70	8.70	25.00	0.80	17.70	22.50	15.00	88.50	9.40	15.40	6.00
29	IC216976	111.00	83.70	5.00	21.70	0.80	19.70	16.50	13.00	83.00	4.30	7.20	3.00
30	IC217016	114.00	92.30	12.70	25.30	0.50	18.30	35.00	50.00	71.50	12.50	18.50	6.00
31	IC217027	112.00	94.00	2.70	18.00	0.60	21.70	23.00	18.00	108.00	5.50	7.90	2.30
32	IC216997	119.00	91.30	9.00	25.70	0.80	17.70	29.00	45.00	90.50	5.50	9.60	4.10
33	IC217026	118.00	60.00	4.30	18.00	0.60	16.30	22.50	23.00	67.50	5.90	9.60	3.70
34	IC217052	107.00	78.70	5.00	22.70	0.70	16.70	20.00	20.00	83.00	6.30	10.90	4.70
35	IC217073	114.00	95.00	4.70	21.30	0.60	19.00	23.50	28.00	95.00	7.90	15.60	7.60

36	IC217075	112.00	96.70	4.70	17.30	0.70	17.00	18.50	33.00	99.50	6.00	11.00	5.00
37	IC217082	112.00	88.00	3.70	18.70	0.70	17.30	25.00	28.00	89.50	11.90	19.70	7.80
38	IC217077	112.00	83.00	3.70	16.70	0.70	17.70	17.50	8.00	80.00	3.70	6.60	2.90
39	IC217107	111.00	85.70	5.00	19.00	0.70	19.70	18.50	23.00	92.00	4.50	6.90	2.40
40	IC217120	112.00	83.00	6.00	19.70	0.60	16.30	24.00	23.00	95.50	5.00	9.30	4.30
41	IC217124	130.00	91.00	8.30	18.00	0.60	18.30	25.50	40.00	87.50	6.00	10.30	4.30
42	IC217275	125.00	102.00	8.30	25.00	0.70	21.70	22.50	43.00	103.00	14.40	22.10	7.80
43	IC217293	111.00	98.00	3.70	20.30	1.00	20.00	25.00	38.00	99.00	7.40	10.50	3.10
44	IC217339	119.00	82.70	3.00	22.70	0.90	20.00	23.00	25.00	89.50	3.80	7.40	3.60
45	IC217345	114.00	92.70	4.30	21.70	0.70	18.70	23.00	20.00	110.00	5.10	8.60	3.40
46	IC217347	119.00	109.00	5.30	25.70	0.60	24.00	25.50	30.00	105.00	12.70	21.10	8.40
47	IC217357	119.00	121.30	7.30	22.00	0.80	22.00	25.00	28.00	129.00	12.80	21.80	9.00
48	IC217366	118.00	105.00	5.70	25.70	0.80	22.00	27.50	38.00	102.00	8.60	14.90	6.40
49	IC217515	111.00	100.00	7.00	24.00	0.90	23.70	24.50	33.00	102.00	4.30	5.70	1.30
50	IC217519	111.00	110.00	5.70	28.00	0.80	23.00	28.00	33.00	120.00	6.60	10.50	3.90
51	IC217566	121.00	89.30	2.30	26.00	0.80	21.30	24.50	25.00	92.50	5.10	9.10	4.00
52	IC217567	121.00	91.70	5.30	20.30	0.90	17.00	24.00	50.00	104.00	6.00	7.20	1.30
53	IC217609	119.00	110.00	6.30	32.30	0.60	23.00	28.50	38.00	114.00	11.70	18.80	7.10
54	IC217646	116.00	90.70	3.30	24.30	0.80	18.30	22.50	30.00	96.50	4.20	8.30	4.10
55	IC217678	118.00	91.30	7.30	26.30	0.80	20.70	18.50	20.00	94.00	5.20	9.90	4.70

56	IC217684	119.00	103.70	5.30	28.00	0.80	23.00	26.50	60.00	102.00	5.20	9.80	4.60
57	IC217680	119.00	96.30	5.30	24.30	0.70	23.00	25.50	50.00	113.00	5.70	9.90	4.20
58	IC217688	118.00	88.00	6.00	28.00	0.90	22.00	22.00	50.00	107.00	3.90	5.30	1.40
	Mean	115.40	90.20	5.70	22.40	0.70	19.10	24.30	30.70	93.00	6.20	10.40	4.20
	Standard Error	0.80	1.60	0.30	0.50	0.00	0.40	0.50	1.68	2.00	0.50	0.80	0.30
	Range	34.00	61.30	10.30	18.70	0.50	10.50	18.50	55.00	81.00	16.60	22.10	9.60
	Max	130	121	12.70	29.50	1.17	24	35	60	129	16.60	22.10	9.60
	Min	100	51	2	13.70	0.50	11	16.50	5	48	0.80	1.60	0.30



Figure 4.1a: Evaluation of germplasm lines with parental lines during Kh17 at low soil P screening plot of ICAR-IIRR



Figure 4.1b: Screening of rice germplasm lines under low p soils and performance of lines growing under low soil P plot at ICAR-IIRR during Kh17

under low P condition is given in the Table 4.1, Figure 4.1a and 4.1b. Analysis of variance revealed the presence of significant variation among the germplasm lines as well as among the checks for all the traits studied, except for flag leaf width (Table 4.2).

The plant height was varied from 60 (IC217026) to 121 (IC217357) cm with a mean of 90.20 cm (Table 4.1). Tissue expansion rate and P status of the tissue growth zone shows direct relation under low soil P condition result in plant height reduction (Cancellier *et al.* 2012; Kavanova *et al.* 2006). Shorter zone of cell division with lower cell production rates under low P condition could be the reason for lower rate of tissue expansion which may in turn lead to reduction in overall growth of plant parts and the plant (Assuero *et al.* 2004).

Among the lines which flowered, days to 50% flowering ranged from 100 (IC216829) to 125 (IC217275) days with a mean of 114.4 days. In comparison to normal soil P condition general delay in flowering was noticed in most of the rice lines under low soil P condition. Phenological delays have been reported for crop plants when exposed to low soil P condition (Shepherd *et al.*, 1987; Rodriguez *et al.*, 1998; Swamy *et al.*, 2019). Delay in flowering and sometimes failure of flowering under P deficient condition in rice was used to identify P deficiency condition (Dobermann and Fairhurst, 2000).

The number of productive tillers among germplasm lines under low P condition varied from 2.30 (IC217566) to 12.70 (217016) with the mean of 5.70. Whereas flag leaf length varies from 13.70 (IC216701) to 32.30 (IC217609) cm with a mean of 22.40 cm and flag leaf width varies from 0.50 (IC216840) to 1 (IC216610, IC216901, IC217293) cm with a mean of 0.70 cm. Panicle length among germplasm lines observed with the mean of 19.10 cm and varies from 13.5 (IC216701) to 24 (IC217347) cm. The shoot length varies from 65 (IC216829) to 129 (IC217357) cm with the mean of 93 cm. The root length among germplasm falls between 16.5 (IC216976) and 35 (IC217016) cm with a mean of 24.30 cm. The grain yield per plant value under low P condition varied from 0.3 (IC216701) to 9.63 (IC216831) g with a mean value of 4.20 g.

The biomass produced under low P condition was in the range of 1.60 to 23.70 g with a mean of 10.37 g. An adaptive mechanism of plants which leads to increased phosphorus acquisition and utilization has been reported to be responsible for tolerance to low soil P (Nord and Lynch, 2008), helping crop plants to attain maximum

Table 4.2: Analysis of variance for growth and yield parameters of the germplasm lines grown under Low soil P plot at ICAR-IIRR, Hyderabad during *Kharif*-2017

Source of variation	df	Days to 50% flowering (Days)	Panicle length (cm)	Root length (cm)	Plant height (cm)	Number of productive tillers/plant (nos.)	Flag leaf length (cm)	Flag leaf width (cm)	Grain yield per plant (g)	Biomass (g)	Dry shoot weight (g)	Shoot length (g)
Block	2	0.08 ^{NS} (0.8249)	0.33 ^{NS} (0.2628)	0.74 ^{NS} (0.6578)	1.84 ^{NS} (0.4599)	0.46 ^{NS} (0.5354)	0.07 ^{NS} (0.7617)	7.01 ^{NS} (0.4437)	0.77 ^{NS} (0.2631)	1.34 * (0.0135)	1.29 ^{NS} (0.5860)	18.98 ^{NS} (0.2646)
Treatments	59	204.74** ($<.0001$)	6.05 ** (0.0016)	13.54 * (0.0242)	236.34 ** (0.0001)	4.23 * (0.0368)	13.55 ** (0.0006)	0.62 ^{NS} (0.99)	9.03 ** (0.004)	31.49 * ($<.0001$)	19.15 * (0.0216)	403.23 * (0.0012)
Tests	56	200.23** ($<.0001$)	5.12 ** (0.0023)	12.40 * (0.0284)	121.07 ** (0.0005)	4.29 * (0.0359)	12.57** (0.0007)	0.23 ^{NS} (0.99)	4.84 * (0.0131)	29.099* ($<.0001$)	12.36 * (0.0476)	141.02 * (0.0095)
Among checks	3	377.37** ($<.0001$)	13.55 ** (0.0006)	5.70 ^{NS} (0.128)	54.92 ** (0.0044)	4.46 * (0.0481)	0.01 ^{NS} (0.9656)	8.26 ^{NS} (0.3953)	76.65 ** (0.0001)	112.07 * ($<.0001$)	199.49* (0.0004)	402.44 * (0.0023)
Test V/s Checks	1	98.00 ** (0.0001)	43.95 ** ($<.0001$)	88.98 ** (0.0017)	6987.48 * ($<.0001$)	0.28 ^{NS} (0.5415)	95.61 ** ($<.0001$)	7.29 ^{NS} (0.365)	101.24 ** ($<.0001$)	3.21 * (0.0038)	30.04 * (0.0196)	15013.18 * ($<.0001$)
Error	4	0.42	0.17	1.59	1.94	0.63	0.24	7.00	0.41	0.088	2.11	10.05
GM		113.85	18.77	23.80	86.01	5.71	21.91	0.87	4.70	10.287	6.444	88.020
CD at 5%Among test		2.54	1.64	4.95	5.47	3.11	1.93	3.74	2.51	1.167	5.707	12.45
CD at 5%Test V/s Checks		2.24	1.44	4.36	4.82	2.74	1.70	3.30	2.21	1.029	5.033	10.98
CV (%)		0.57	2.22	5.29	1.62	13.86	2.25	303.58	13.59	2.89	22.55	3.60

reproductive biomass. The crop P efficiency can be defined as the ability to produce biomass or yield under certain available P supply conditions (Wissuwa *et al.* 2009) and it can also be further explained as the capability of P uptake from the available source and tolerance in P insufficient conditions (Wissuwa and Ae 2001; Wissuwa 2005; Wang *et al.* 2010).

The phenotypic variability noticed for different productivity related traits under low soil P condition, implies that there is a definite possibility for improving rice cultivars under low P condition (Fageria *et al.*, 1988a; Akinrinde and Gaizer, 2006; Aluwihare *et al.*, 2016). In our study, significant differences were observed among the tolerant and sensitive checks in the low soil P plot; such differences were not obvious in the normal soil P plot (Table 4.1 and Table 4.3). Under low P condition, reduction in plant height, tiller number, flag leaf length, flag leaf width, root length and grain yield was observed in most of the lines. Under the normal condition, all the rice lines flowered and the flowering was early as compared to low P condition as under normal soil P condition, the plants were not starved for P and sufficient P was acquired in a short period of time due to abundant soil P. Presence of significant variation for these traits, among the germplasm lines analysed the results indicated the potentiality of the germplasm lines in terms of contributing in terms of known source (i.e. *Pup1* or its novel alleles) and also novel sources of tolerance (i.e. non-*Pup1* type tolerance). The results obtained from the present study revealed the presence of variability among the germplasm lines for important agro-morphological traits under low soil P condition and provides a possibility for improving rice cultivars for low soil P using some of the germplasm lines as donors.

The germplasm lines were also evaluated under sufficient soil P (i.e. normal) condition. Significant difference was observed among the germplasm lines for all the traits studied (Table 4.3). The days to fifty percent flowering varied between 91 (IC216909) and 106 (IC217073) days. The plant height ranged from 130 (IC217027) to 160 (IC216806) cm. The number of productive tillers ranged between 5.25 and 10.75 (IC217124) tillers per plant. Flag leaf length under normal condition varied from 20 (IC216736) to 43 (216767) cm and flag leaf width ranged between 1 and 1.6 cm. The grain yield per plant varied from 5.1 (IC216686) to 37.5 (IC217120) g. The thousand grains weight ranged from 19.2 (IC216934) to 26.66 (IC217347) g.

Table 4.3: Performance of rice germplasm lines in normal soil P condition at ICAR-IIRR, during *Kharif*, 2017

SI. no	Germplasm lines	Days to 50% flowering (Days)	Plant height (cm)	Number of productive tillers/plant (No)	Leaf length (cm)	Leaf width (cm)	Grain yield/Plant (g)	Thousand grain weight. (g)
1	Swarna	122	81	10	22.5	1.39	27	20.5
2	ISM	103	85	9	24.17	1.38	21	14.62
3	IC216610	95	144	7	42	1.2	19.2	2.54
4	IC216682	97	150	8	24	1	19.3	2.62
5	IC216686	93	144	6	26	1	5.1	2.58
6	IC216736	94	158	7	20	1	20.9	2.22
7	IC216739	106	152	5	27	1	7	2.48
8	IC216701	98	155	9	27	1	8.6	2.38
9	IC216731	106	152	5	34	1	29.7	2.42
10	IC216767	96	160	7	43	1.4	16.1	2.32
11	IC216819	92	146	7	41	1.3	12.9	2.48
12	IC216823	98	135	10	37	1.3	12	2.34
13	IC216798	100	140	8	43	1.6	13	2.2
14	IC216820	94	145	10	29	1	17	2.47
15	IC216829	96	147	6	31	1	14.4	2.2
16	IC216806	93	160	10	37	1.2	14.5	2.57
17	IC216810	95	140	8	41	1	11.1	2.6
18	IC216831	104	146	10	34	1.3	15.2	2.5
19	IC216840	98	154	9	33	1.4	11	2.36
20	IC216938	92	155	8	34	1.6	13.7	2.24
21	IC216901	93	150	8	34	1.5	15.8	2.22
22	IC216903	96	160	9	31	1.4	13.8	2.62
23	IC216909	95	156	8	28	1.3	14.4	2.28
24	IC216934	91	152	7	42	1.6	15	2.58
25	IC216948	92	145	7	28	1	16.3	1.92
26	IC216956	103	153	8	29	1	24.3	2.44
27	IC216957	97	157	7	32	1	13	2.64
28	IC216942	105	100	10	27	1	14.4	2.44
29	IC216976	93	150	9	31	1	13.7	2.23
30	IC217016	99	132	7	27	1	14.4	2.42
31	IC217027	93	130	8	23	1	10	2.44
32	IC216997	91	136	5	31	1	13.7	2.52
33	IC217026	97	140	7	23	1	9.3	2.6
34	IC217052	98	135	7	34	1.5	21.1	2.28
35	IC217073	106	140	7	34	1.5	16.2	2.2

36	IC217075	96	125	6	43	1.2	16.1	2.24
37	IC217082	98	136	8	37	1.4	10.1	2.22
38	IC217077	97	130	9	41	1.6	22.1	2.23
39	IC217107	100	155	7	33	1.5	34	2.48
40	IC217120	98	138	9	41	1.3	37.5	2.54
41	IC217124	100	136	11	24	1	20.2	2.36
42	IC217275	98	145	9	22	1	10.3	2.26
43	IC217293	97	130	10	23	1	20.8	2.54
44	IC217339	98	158	8	23	1	22.2	2.54
45	IC217345	93	154	9	24	1	18.1	2.62
46	IC217347	91	150	10	24	1	27.2	2.66
47	IC217357	105	146	9	22	1	33.7	2.44
48	IC217366	97	140	9	28	1.5	24.9	2.32
49	IC217515	98	145	7	28	1	25.3	2.44
50	IC217519	96	142	8	31	1	18.1	2.36
51	IC217566	102	135	8	34	1.4	9.5	2.5
52	IC217567	105	140	8	36	1.5	23.7	2.48
53	IC217609	106	151	6	37	1.5	15.5	2.26
54	IC217646	98	143	7	33	1.4	23.5	2
55	IC217678	94	146	7	31	1.5	20.2	2.62
56	IC217684	100	125	8	34	1.3	21.1	2.54
57	IC217680	105	145	10	31	1.4	14.4	2.54
58	IC217688	105	147	6	36	1.5	17.6	2.58

4.1.1.2 Estimation of phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV), heritability and genetic advance under low P condition

Estimation of phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) revealed the presence of high variability for the traits studied for low soil P tolerance. PCV and GCV obtained, were categorized as low (0-10%), moderate (10-20%) and high (>20%) as indicated by Sivasubramanian and Madhavamenon (1973). The PCV values ranged from 3.07 % (panicle length) to 190.82 % (Number of tillers). Low PCV value was noticed for panicle length (3.07 %), medium PCV values were observed for days to fifty percent flowering (12.50 %), grain yield per plant (11.19). Rest of the traits viz., plant height (54.95 %), number of tillers (190.82 %), root length (77.23 %) and flag leaf length (20.07 %) exhibited high PCV values (Table 4.4). The GCV values were varied from 3.01 % (panicle length) to 176.35 % (number of productive tillers). Low GCV values were observed for panicle length (3.01 %) while medium GCV values were observed for grain yield per plant (10.71%), days to 50% flowering (12.49 %) and flag leaf length (19.88 %). High GCV values were found for the traits viz., plant height (54.51%), number of tillers (176.35 %), biomass (55.72 %) and root length (72.12 %) (Table 4.4).

The GCV and PCV provide a measure to compare the variability present among the traits. Results obtained in the study are in accordance with Fukrei *et al.* (2011), Ameen *et al.* (2016), Abebe *et al.* (2017) and Onyia *et al.* (2017). Values of PCV and GCV were close for most of the traits except for number of tillers and root length indicating their stable nature. Similar findings were reported by Rao *et al.* (1996). Selection based on phenotypic performance of these characters would be effective to bring the considerable genetic improvement in them under low P condition. In the present study, the traits viz., plant height, number of productive tillers and root length possessed high GCV and PCV values among which plant height showed small difference between GCV and PCV values and further these traits were used indicator for low P condition in several studies (Fageria *et al.*, 1988a, 1988b; Dobermann and Fairhurst, 2000; Wissuwa and Ae, 2001; Wissuwa, 2005; Li *et al.*, 2009; Jakkeral and Kajjidoni, 2011; Chiangmai and Yodmingkhwon 2011; Zhou *et al.*, 2016; Tian *et al.*, 2017 ; Swamy *et al.* 2019) which indicate that these traits could be more useful in improvement of rice under low soil P condition.

The broad sense heritability (ratio of genotypic variance to phenotypic variance) is generally expressed in percent, which represent the heritable portion of phenotypic variance and gives an idea about the exploitable portion of variation. It is a good index of the transmission of characters from parents to their progenies (Falconer, 1989) and can be used as a predictor of improvement/gain under selection, in the selection procedures (Allard, 1960). In the present study broad sense heritability estimates (Table 4.4) varied from 85.42 (number of tillers) to 99.79% (days to 50% flowering). All the traits viz., number of tillers (85.42 %), flag leaf length (98.08%), panicle length (96.60 %), grain yield per plant (91.57 %), plant height (98.40 %), days to 50% flowering (99.79 %), showed broad sense heritability. These results are in accordance with the results of Fukrei *et al.* (2011), Ameenul *et al.* (2016), Abebe *et al.* (2017) and Onyia *et al.* (2017). High broad sense heritability estimates doesn't always mean that it will give high genetic advance or gain. Because, here the heritability estimates involves both additive and non-additive portion of genetic variance. Non-additive portion of the genetic variance is predominant within population, even though broad sense heritability values are high, genetic gain through the selection will reduce. So, Johnson *et al.* (1955) suggested that heritability estimates and genetic advance as percent of mean together would provide a better judgement rather than heritability alone in predicting the resultant effects of selection.

Genetic advance as percent of mean estimates for the traits studied under the present study (Table 4.4) were ranged from 10.18 (root length) to 528.13 % (flag leaf width). Traits like days to 50% flowering (25.69 %), flag leaf length (40.56 %), grain yield per plant (41.60 %), plant height (111.38 %), number of tillers (335.75 %) and Root length (138.73 %) processed high genetic advance as percent mean. Whereas panicle length (6.10 %) registered low genetic advance as percent mean. Similar results were found by Yadav *et al.* (2010), Fukrei *et al.* (2011), Ameenul *et al.* (2016) and Abebe *et al.* (2017). The overall results indicated that the germplasm lines studied were having high genetic variability with high genetic advance as percent mean for all the traits studied except for panicle length. Selection based on these traits would be very effective in improving the rice lines for low soil P tolerance.

Table 4.4: PCV, GCV, Heritability and Genetic advance for different traits of germplasm lines grown under low soil P plot at ICAR-IIRR, during *Kharif*, 2017

Trait	Coefficient of Variation			Heritability %	Genetic Advance	Genetic advance as % of mean
	PCV%	GCV %	ECV %			
Days to 50% flowering (Days)	12.50	12.49	0.57	99.79	29.09	25.69
Panicle length (cm)	3.07	3.01	0.57	96.60	4.50	6.10
Root length(cm)	77.23	72.12	27.64	87.19	6.33	138.73
Plant height(cm)	54.95	54.51	6.96	98.4	22.3	111.38
Number of productive tillers /Plant (nos)	190.82	176.35	72.87	85.42	3.64	335.75
Flag leaf length	20.07	19.88	2.78	98.08	7.16	40.56
Grain yield per plant	11.19	10.71	3.25	91.57	8.18	41.60

4.1.1.3 Assessment of stress tolerance indices among the rice germplasm based on their performance in low soil P and normal P soils:

Several criteria have been developed and used by breeders to aid the selection process. One such criterion is selection of genotypes based on their performance in stress and non-stress environments through many selection indices (Clarke *et al.*, 1992). Several indices have been used for assessment of drought stress tolerance in many crops (Mollasadeghi *et al.*, 2011; Ashraf *et al.*, 2015). In the present study stress indices like, stress tolerance index (STI; Fernandez, 1992), tolerance index (TOL; Rosielle and Hamblin, 1981), yield reduction ratio (YR, Golestani–Araghi and Assad, 1998), stress susceptibility index (SSI; Fisher and Maurer, 1978), yield stability index (YSI; Bouslama and Schapaugh, 1984), yield index (YI; Gavuzzi *et al.*, 1997) and percent yield reduction (PYR) were calculated on the basis of grain yield under normal (under recommended dose of P i.e. 60 kg P ha⁻¹) condition and grain yield under low soil P (< 2 kg ha⁻¹; low P stress) condition. These indices were used by several workers for drought tolerance assessment, salinity tolerance, low nitrogen or nitrogen deficiency tolerance in many crops (Mollasadeghi *et al.*, 2011; Ashraf *et al.*, 2015; Singh *et al.*, 2015), (Rameeh, 2015; Khan and Mohammad, 2016). Reports on use of these indices for assessing low P tolerance is limited (Swamy *et al.*, 2019). In the present study an effort was made to assess the low P tolerance using above mentioned indices, and the results found were given in the Table 4.5 and summarized as follows.

Stress tolerance index (STI) is directly proportional to tolerance level; hence genotypes with high STI values are expected to be more tolerant (Fernandez, 1992; Ashraf *et al.*, 2015). The STI value was highest for Swarna (0.68) followed by IC216731 (0.66) and IC216831 (0.47) whereas minimum values were observed for IC216701, IC216819, IC216829 and IC216840 (0 to 0.6), while the sensitive check, Improved Samba Mahsuri (ISM) recorded a very low value of 0.30.

Yield stability index (YSI) has direct proportion to tolerance level thus stable genotypes under stress and normal conditions has high value of YSI as compared to sensitive genotypes (Bouslama and Schapaugh, 1984; Singh *et al.* 2015). The highest value for yield stability index (YSI) was found for IC217082 (0.80), IC216686 (0.72) and IC216831 (0.61), followed by the tolerant check, Swarna, which recorded a value of

Table 4.5: Stress indices calculated for germplasm lines based on single plant yield under normal and low soil P conditions

Genotype	Grain yield under normal plot (g)	Grain yield under low Soil P plot (g)	STI/ Stress tolerance index	TOL/ tolerance	YR/ yield reduction ratio	SSI/ stress susceptibility index	YSI/ yield stability index	YI/ Yield index	PYR/ Percent yield reduction
Swarna	26.89	9.00	0.60	17.85	0.65	0.79	0.35	2.75	66.48
ISM	18.27	5.07	0.30	13.20	0.72	0.98	0.28	1.15	72.24
IC216610	19.32	2.76	0.18	16.56	0.86	1.16	0.14	0.63	85.71
IC216682	19.42	3.03	0.19	16.39	0.84	1.14	0.16	0.69	84.41
IC216686	7.42	5.35	0.13	2.07	0.28	0.38	0.72	1.22	27.91
IC216736	21.02	3.66	0.25	17.36	0.83	1.12	0.17	0.83	82.58
IC216739	7.12	2.63	0.06	4.49	0.63	0.85	0.37	0.60	63.10
IC216701	8.72	0.04	0.00	8.68	1.00	1.35	0.00	0.01	99.56
IC216731	29.82	6.76	0.66	23.06	0.77	1.04	0.23	1.54	77.33
IC216767	16.22	1.59	0.09	14.63	0.90	1.22	0.10	0.36	90.17
IC216819	13.02	0.31	0.01	12.72	0.98	1.32	0.02	0.07	97.66
IC216823	12.12	2.46	0.10	9.66	0.80	1.08	0.20	0.56	79.69
IC216798	13.12	3.39	0.15	9.73	0.74	1.00	0.26	0.77	74.13
IC216820	17.12	2.96	0.17	14.16	0.83	1.12	0.17	0.67	82.70
IC216829	14.52	0.46	0.02	14.06	0.97	1.31	0.03	0.10	96.82
IC216806	14.62	5.09	0.24	9.53	0.65	0.88	0.35	1.16	65.16
IC216810	11.22	2.39	0.09	8.83	0.79	1.06	0.21	0.54	78.66
IC216831	15.32	9.29	0.47	6.03	0.39	0.53	0.61	2.11	39.34
IC216840	11.12	0.66	0.02	10.46	0.94	1.27	0.06	0.15	94.05
IC216938	13.59	6.88	0.31	6.70	0.49	0.67	0.51	1.56	49.34
IC216901	15.69	5.42	0.28	10.27	0.65	0.88	0.35	1.23	65.47
IC216903	13.69	5.32	0.24	8.37	0.61	0.83	0.39	1.21	61.15
IC216909	14.29	3.18	0.15	11.10	0.78	1.05	0.22	0.72	77.72
IC216934	14.89	5.75	0.28	9.14	0.61	0.83	0.39	1.31	61.37
IC216948	16.19	2.58	0.14	13.60	0.84	1.14	0.16	0.59	84.04
IC216956	24.19	2.78	0.22	21.40	0.88	1.20	0.12	0.63	88.49
IC216957	12.89	3.05	0.13	9.84	0.76	1.03	0.24	0.69	76.33
IC216942	14.29	6.22	0.29	8.07	0.56	0.76	0.44	1.41	56.49
IC216976	13.59	3.22	0.14	10.37	0.76	1.03	0.24	0.73	76.32
IC217016	14.29	6.28	0.30	8.00	0.56	0.76	0.44	1.43	56.02
IC217027	9.89	2.58	0.08	7.30	0.74	1.00	0.26	0.59	73.87

Genotype	Grain yield under normal plot (g)	Grain yield under low Soil P plot (g)	STI/ Stress tolerance index	TOL/ tolerance	YR/ yield reduction ratio	SSI/ stress susceptibility index	YSI/ yield stability index	YI/ Yield index	PYR/ Percent yield reduction
IC216997	13.59	4.35	0.19	9.24	0.68	0.92	0.32	0.99	67.98
IC217026	9.19	3.98	0.12	5.20	0.57	0.77	0.43	0.91	56.64
IC217052	20.99	4.92	0.34	16.07	0.77	1.03	0.23	1.12	76.57
IC217073	16.32	7.29	0.39	9.03	0.55	0.75	0.45	1.66	55.31
IC217075	15.99	5.28	0.28	10.70	0.67	0.90	0.33	1.20	66.95
IC217082	9.99	8.02	0.26	1.97	0.20	0.27	0.80	1.82	19.73
IC217077	21.99	3.12	0.23	18.87	0.86	1.16	0.14	0.71	85.82
IC217107	33.89	2.65	0.30	31.24	0.92	1.25	0.08	0.60	92.18
IC217120	37.49	4.35	0.54	33.13	0.88	1.19	0.12	0.99	88.39
IC217124	20.19	4.39	0.29	15.80	0.78	1.06	0.22	1.00	78.27
IC217275	10.29	7.85	0.27	2.43	0.24	0.32	0.76	1.79	23.66
IC217293	20.79	3.15	0.22	17.63	0.85	1.15	0.15	0.72	84.83
IC217339	22.19	3.72	0.27	18.47	0.83	1.12	0.17	0.85	83.23
IC217345	18.09	3.52	0.21	14.57	0.81	1.09	0.19	0.80	80.54
IC217347	27.19	8.49	0.76	18.70	0.69	0.93	0.31	1.93	68.78
IC217357	33.69	9.05	1.00	24.63	0.73	0.99	0.27	2.06	73.12
IC217366	24.89	6.45	0.53	18.43	0.74	1.00	0.26	1.47	74.07
IC217515	25.29	1.42	0.12	23.87	0.94	1.28	0.06	0.32	94.38
IC217519	18.09	3.95	0.24	14.13	0.78	1.06	0.22	0.90	78.14
IC217566	9.49	4.09	0.13	5.40	0.57	0.77	0.43	0.93	56.92
IC217567	23.69	1.35	0.11	22.33	0.94	1.27	0.06	0.31	94.28
IC217609	15.49	7.19	0.37	8.30	0.54	0.72	0.46	1.63	53.60
IC217646	23.49	4.22	0.33	19.27	0.82	1.11	0.18	0.96	82.03
IC217678	20.19	4.75	0.32	15.43	0.76	1.03	0.24	1.08	76.45
IC217684	21.09	4.65	0.32	16.43	0.78	1.05	0.22	1.06	77.93
IC217680	14.39	4.29	0.20	10.10	0.70	0.95	0.30	0.97	70.20
IC217688	17.59	1.52	0.09	16.07	0.91	1.23	0.09	0.35	91.35

0.55. The lowest value were found for the genotypes IC216701 (00.0), IC216819 (0.02) and IC216829 (0.03). The sensitive check ISM showed value of 0.28.

As yield index (YI) is directly correlated to tolerance level, the genotypes with high value of YI will be suitable for stress conditions (Gavuzzi *et al.*, 1997; Ashraf *et al.*, 2015; Singh *et al.*, 2015). The highest value of yield index (YI) was recorded for IC216831 (2.11) followed by IC217082 (1.82) with positive check Swarna (2.43). The lowest value was observed in the genotypes IC216701 (0.01), IC216819 (0.07) and IC216829 (0.10). The sensitive check, ISM showed value of 1.15.

Tolerance index (TOL) values show inverse relationship for stress tolerance. Hence, rice lines with lower value of this index are more stable under both P deficient and P sufficient conditions (Rosielle and Hamblin, 1981; Singh *et al.*, 2015). The lowest value was recorded for the genotypes IC217082 (1.97), IC216686 (2.07), IC216739 (4.49) followed by positive check Swarna (8.83) and IC216831 (6.03). It was highest for genotypes IC216731 (23.06), IC216956 (21.40) and IC216610 (16.56), while the sensitive checks ISM recorded a value 13.20. In the present study, Swarna a low P tolerant check lower TOL values in comparison with the sensitive check ISM, while IC216831 was found to have lower TOL value of 6.03 that indicates its tolerance ability for low soil P condition.

The stress index parameter, yield reduction ratio (YR) is also inversely proportional to stress tolerance level. The lowest yield reduction ratio (YR) was observed for the genotypes IC216686 (0.28) and IC217082 (0.20), while the tolerant check, Swarna recorded a value of 0.45. The highest yield reduction value was found for IC216701 (1.00), IC216819 (0.98) and IC216829 (0.97), while the sensitive check, ISM recorded a value of 0.72. In present study, low soil P tolerant check Swarna and IC216831 possessed lower YR value as compared to the sensitive check, ISM. The genotypes which are having low value of YR are more suitable for low soil P conditions (Golestani-Araghi and Assad, 1998; Ashraf *et al.*, 2015). The percent yield reduction under low P condition was observed to be highest for the genotypes IC216701 (99.56), IC216819 (97.66) and IC216829 (96.82) and the tolerant check Swarna recorded a lower value of 45.26. The lowest percent yield reduction value recorded for some of the rice lines, viz., IC217082 (19.73), IC216686 (27.91) and IC216831 (39.24). The sensitive check, ISM showed a percent yield reduction of 72.24 under low P condition.

The tolerant check Swarna and germplasm line IC216831 had low percent yield reduction values in comparison to sensitive check ISM, indicating that IC216831 is a tolerant genotype.

The genotypes with stress susceptibility index (SSI) values less than one will be more tolerant under stress condition (Fisher and Maurer, 1978; Ashraf *et al.*, 2015). Lowest stress susceptibility index (SSI) values were noted for IC217082 (0.27), IC216686 (0.38) and IC216831 (0.53), followed by the positive check, Swarna (0.45). High values of SSI were recorded for IC216701 (1.35), IC216819 (1.32) and IC216829 (1.31). The sensitive check, ISM recorded a value of 0.72. The low P tolerant check, Swarna recorded stress susceptibility index (SSI) value below one (0.61) and IC216831 showed a value of 0.53 that indicate IC216831 has got good tolerance ability to low soil P.

The various stress indices were helpful in selecting genotypes with high yield under stress, but had failed to select genotypes with good yield under both environments. The genotype, IC217082 gave good yield under stress condition while the yield under the normal condition was poor in comparison to other tolerant germplasm lines. Even though they behave similar, in ranking genotypes, different indices will indicate different abilities of the genotype. YSI describes about plasticity of the genotype in stress and normal environment for yield, while SSI, express genotypes extent of sensitivity for the stress, TOL express on the difference in yield in stress and non-stress environment. The different indices classified the different genotypes into different categories with respect to low soil P tolerance, thus leading to contradiction in selection of genotypes, based on single criterion.

The cluster analysis sequestrates genotypes into clusters which exhibit high homogeneity within a cluster and high heterogeneity between clusters (Jaynes *et al.*, 2003). A cluster analysis using the stress tolerance indices, grain yield under normal condition and grain yield under low P condition was performed to group the rice genotypes under study. The dissimilarity matrix was constructed using Euclidean distance, the clustering was performed using Unweighted Pair Group Method using Arithmetic means (UPGMA) through DARwin6.0 software and presented in the Figure 4.2. Similar kind of clustering pattern was found in respect to salinity tolerance and nitrogen tolerance and low soil P tolerance (Singh *et al.*, 2015; Khan and Mohammad, 2016; Swamy *et al.*, 2019).

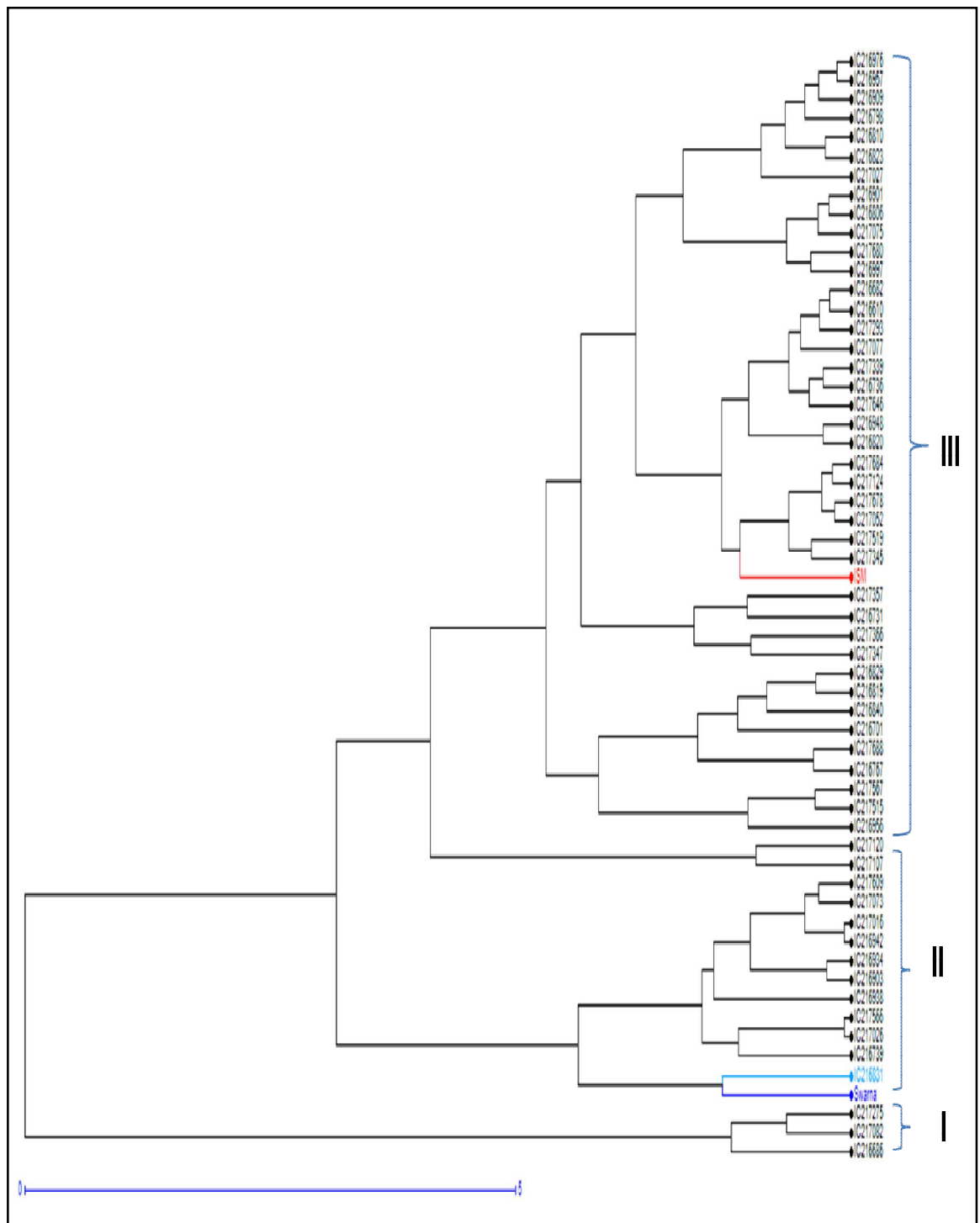


Figure 4.2: Clustering pattern of germplasm lines based on stress indices under low P (< 2 kg P ha⁻¹) and normal P (60 kg P ha⁻¹) soils

In order to get better and combined interpretation of the results obtained and also to overcome the contradictions in selection of tolerant genotypes by using single criterion, a cluster analysis was carried out to group the genotypes behaving similarly in response to low soil P stress. The genotypes were clustered into three major group's viz., highly tolerant, tolerant and sensitive (Figure 4.2) genotypes. The first group which showed <30% yield reduction consisted of three genotypes (IC216686, IC217275 and IC217082). The second group had fourteen genotypes (Swarna, IC216831, IC216739, IC217026, IC217566, IC216938, IC216903, IC216934, IC216942, IC217016, IC217073, IC217609, IC217107 and IC217120), which showed 45% to 65% percent yield reduction. Further, the genotypes in the second group (IC216831 and twelve genotypes) are well suited for use as donor for low P tolerance, as they are good yielders under normal condition. Hence, they could serve as good donors for low soil P tolerance. Forty genotypes showed more >70% yield reduction fall under third group, the third group also included the low soil P sensitive check, Improved Samba Mahsuri.

4.1.2 Molecular characterization of germplasm lines for *Pup1* locus and their cluster analysis

A total of 56 germplasm rice lines were analyzed in the present study in order to determine how many of them possess the tolerant allele with respect to the major gene/QTL associated with low soil P tolerance, *Pup1* (Wissuwa *et al.*, 2002; Chin *et al.*, 2011). The rice lines along with the tolerant (Swarna) and sensitive check (Improved Samba Mahsuri) were genotyped using *Pup1* locus specific dominant markers viz., K46-1, K46-2, K52 and 46CG-1 (Chin *et al.*, 2011; Pariasca-Tanaka *et al.*, 2014). Marker assisted characterization of genotypes for *Pup1* locus based on *Pup1* specific markers revealed that most of the genotypes possess either the partial or full set of sequences associated with *Pup1* locus-specific markers (Figure 4.3). In case of the tolerant check (viz., Swarna), the '+' allele (i.e. the tolerant allele) was present and in the case of the sensitive check (viz., Improved Samba Mahsuri), '-' allele (i.e. the sensitive allele) was present with respect to all four *Pup1*-specific markers. Among the rice lines, the '+' allele (i.e. tolerant allele) specific for the marker K46-2 was observed to be highest (64%), followed by 46CG-1 (62%), K46-1 (55%) and K46-52 (50%). A total of 20 genotypes possessed the tolerant allele for all the four marker loci and nine genotypes (IC216610, IC216731, IC216767, IC216831, IC216903, IC217339, IC21678,

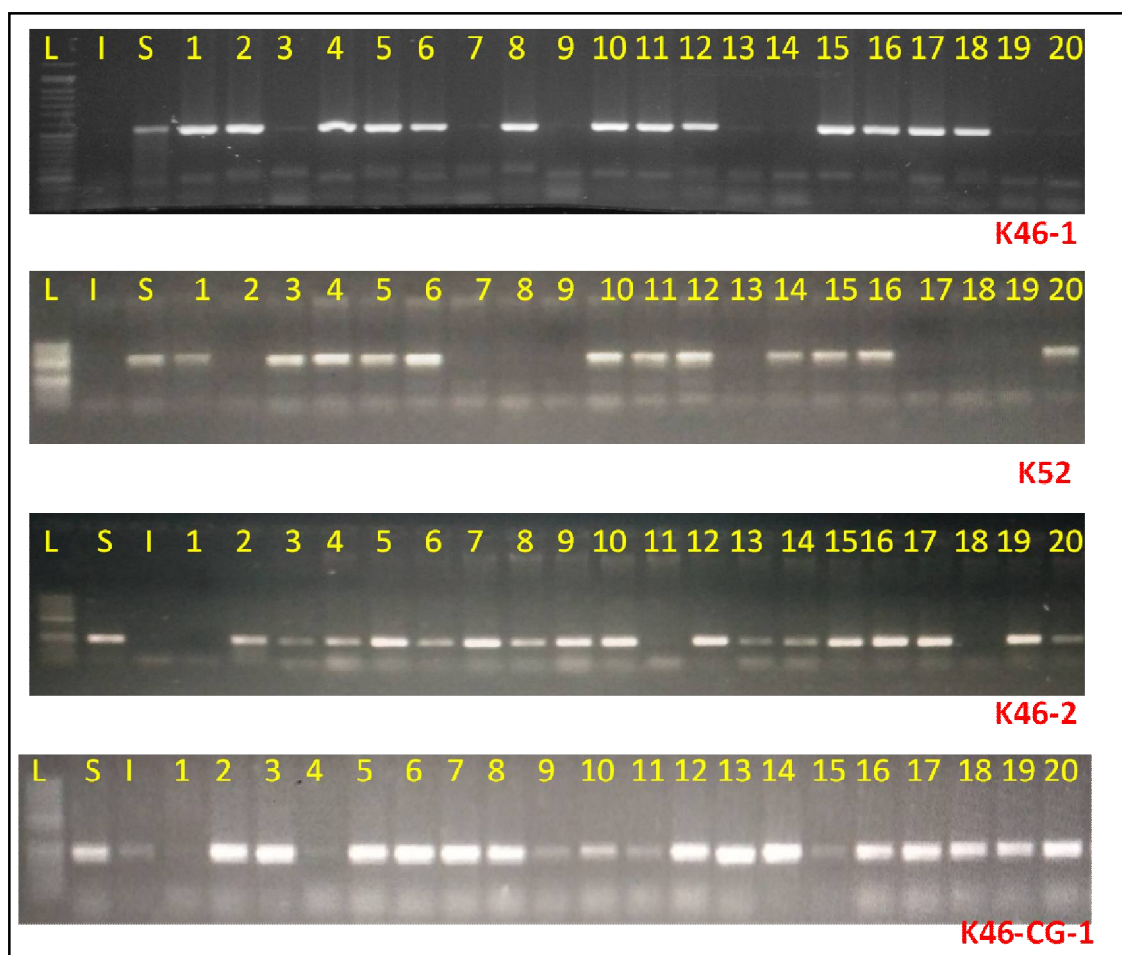


Figure 4.3: Genotyping of rice germplasm lines using *Pup1* locus specific markers. L -100 bp ladder, I- ISM, S- Swarna, 1 to 20 – Germplasm lines used for screening

IC217567 and IC217684) possessed '-' allele (i.e. susceptible allele) with respect to all the four *Pup1* specific markers.

Based on the presence or absence of respective *Pup1* associated markers, the genotypes were grouped by cluster analysis. Among the nine clusters formed, the first group in the cluster had ten genotypes (nine germplasm lines and one low soil P sensitive check, Improved Samba Mahsuri), which were completely devoid of *Pup1*, and possessed '-' allele for all the *Pup1* specific marker loci screened. The ninth group was the biggest group formed with 21, genotypes (Twenty germplasm lines and one low soil-P tolerant checks Swarna) and they all possessed '+' allele for all the *Pup1* specific marker loci analyzed. Remaining clusters were composed of genotypes with various combinations with respect to presence and absence for the *Pup1* associated marker loci (Table 4.6). These results are in congruence with results obtained by Chin *et al.* (2010), Pariasca-Tanaka *et al.* (2014) and Swamy *et al.* (2019) in the germplasm survey for *Pup1* locus using *Pup1* specific markers.

4.1.3 Identification of novel source for low P tolerance

The rice line, IC216831 was completely devoid of *Pup1*, showed significantly higher level of tolerance to low soil P, in comparison to the sensitive genotypes. IC216831, recorded higher value for stress indices, STI, YSI and YI, revealing its tolerance to low soil P and all the sensitive genotypes recorded very low values for these indices. Low value for stress susceptibility index (SSI) (i.e. < 1) indicates that IC216831 is a tolerant genotype. It can be speculated that it may possess novel genomic loci associated to low soil P tolerance and hence could serve as a novel source for low soil P tolerance after dissection of the genetic basis of tolerance and molecular mapping of the tolerance loci (Swamy *et al.*, 2019).

4.2. To study the inheritance of Wazuhophek for low soil P tolerance

4.2.1 Genetic analysis of the parents Wazuhophek and Improved Samba Mahsuri

Swamy *et al.* (2019) screened many rice germplasm lines along with positive (i.e. Swarna, Vandana) and negative checks (Improved Samba Mahsuri, MTU1010 and IR64) for low P tolerance through phenotypic and genotypic analysis. A total of 18 lines

Table 4.6: Clustering of rice germplasm lines into different groups based on *Pup1* specific markers analysis

SI. No.	Groups	allele for the <i>Pup1</i> Specific marker/markers combinations	No. genotypes	List of the genotypes in the Group
1	Group 1	NIL	9 +2**	**ISM,IC216610,IC216731,IC216767,IC216831,IC216903,IC217339,IC217567,IC217678, IC217684
2	Group 2	k46-2	10	IC216957, IC216934, IC216840, IC216976, IC217027, IC217077, IC217357, IC217366,IC217519, IC217124
3	Group 3	46cg1	3	IC16909, IC217073, IC217075
4	Group 4	46-1 and 46-2	1	IC217016
5	Group 5	46-1 and k52	1	IC217566
6	Group 6	46-2 and 46cg1	3	IC217646, IC217293, IC217052
7	Group 7	46-1, k52 and 46cg-1	7	IC217688, IC217609, IC217120, IC217026, IC216956, IC216829, IC216682
8	Group 8	46-1 and 46-2 and 46cg-1	2	IC216739, IC216819
9	Group 9	46-1 and 46-2, k52 and 46cg-1	20+1##	##Swarna,IC216686,IC216736,IC216701,IC216823,IC216798,IC216820,IC216806,IC216810, IC216938,IC16901,IC216948,IC216942,IC216997,IC217082,IC217107,IC217275,IC217345,IC2173 47, IC217515, IC217680

were found to be tolerant to low soil P based on phenotypic analysis. The genotypic analysis of these lines revealed the presence of *Pup1* locus in complete or partial form in all the tolerant genotypes except Wazuhophek. Hence, in the present study, an attempt was made to analyze the inheritance of low soil P tolerance associated traits in Wazuhophek by crossing it with Improved Samba Mahsuri, a cultivar, which is highly sensitive to low soil P and devoid of the *Pup1* gene/QTL (Anila *et al.*, 2017).

4.3. To generate mapping populations for characterizing low soil P tolerance

4.3.1 Development of mapping population

For any trait mapping study, it is ideal to cross parents which are not only contrasting for the trait of interest, but also which are significantly divergent. In this study, a landrace known as Wazuhophek from north eastern part of India that showed low P tolerance (Swamy *et al.*, 2019) used as male parent, while Improved Samba Mahsuri (which is low soil P sensitive) used as female parent. A mapping population consisting of 112 RILs was developed from a cross between Wazuhophek and ISM by single seed descent method and maintained at IIRR, Rajendranagar, Hyderabad (Figure 4.4)

4.4. To tag and map the genomic regions (genes/ QTL) associated with low soil P tolerance using molecular marker analysis

4.4.1 Parental polymorphism survey using SSR markers

4.4.2 Phenotypic evaluation of RILs population for low soil P tolerance

4.4.2.1 Phenotypic evaluation of RILs population in low soil P and normal P soil condition

4.4.2.2 Frequency Distribution of low P tolerance related traits

4.4.2.3 Correlation analysis between traits under study

4.4.3 Genotyping of RILs population using polymorphic markers and development of linkage map with polymorphic SSR markers

4.4.4 Mapping of QTLs using RILs, screened for the low soil P tolerance

4.4.5 *In-silico* identification of the genes present within QTL intervals

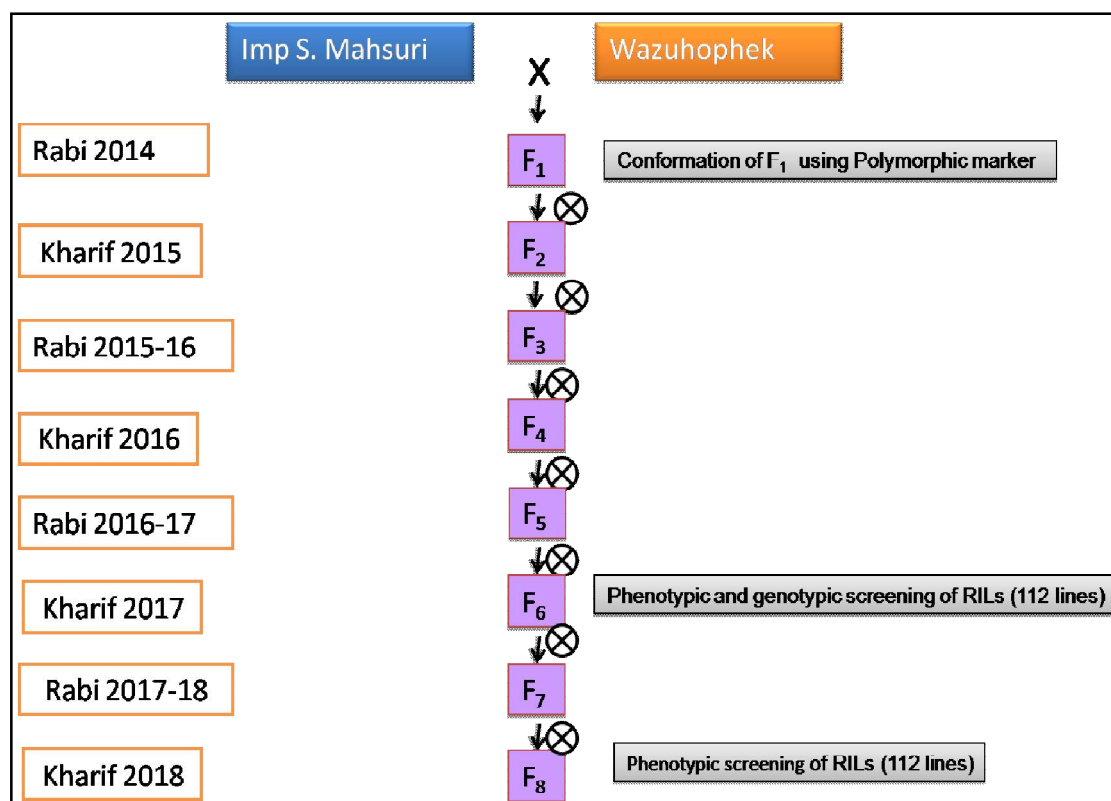


Figure 4.4: Schematic representation of crossing program for development of RIL mapping population (Improved Samba Mahsuri X Wazuhophek)

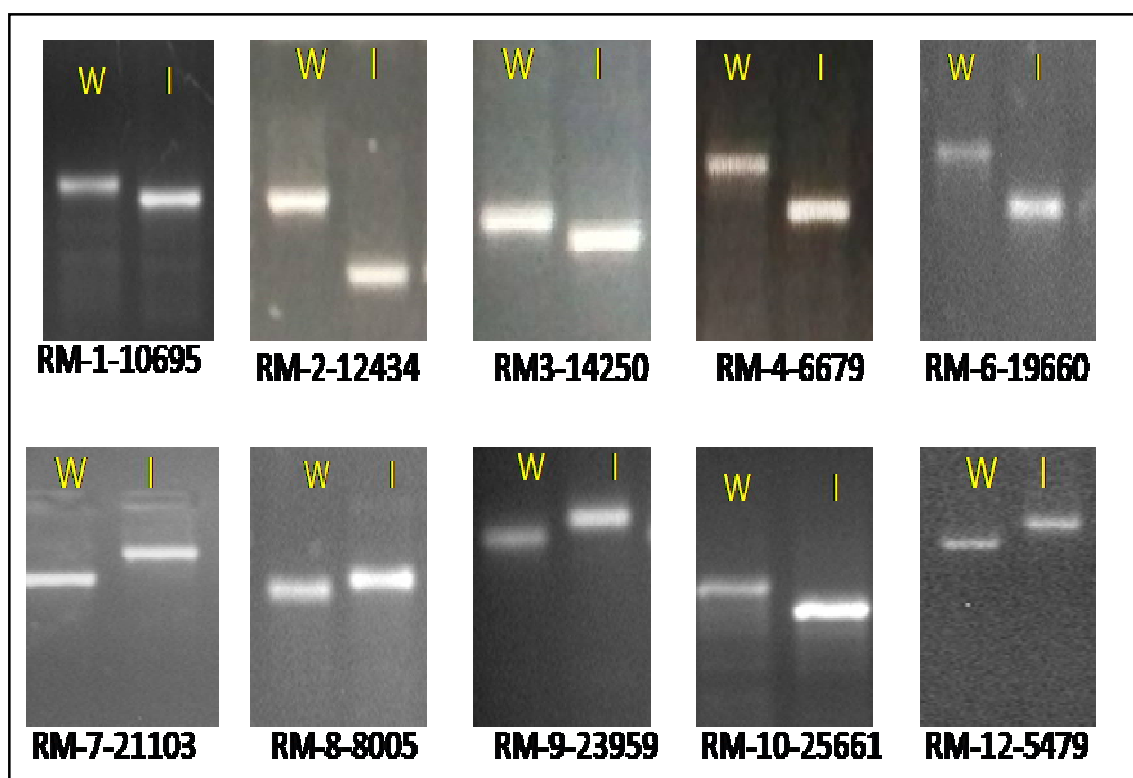


Figure 4.5: Parental Polymorphism analysis between Wazuhophek (W) and Improved Samba Mahsuri (I)

Table 4.7: Chromosome wise list of SSR markers used and polymorphic SSR markers identified for QTL mapping

SI. No.	Chromosome number	Number of polymorphic marker	Total number of markers screened
1	Chr#1	11	49
2	Chr#2	7	47
3	Chr#3	10	47
4	Chr#4	5	51
5	Chr#5	4	43
6	Chr#6	6	56
7	Chr#7	9	51
8	Chr#8	4	49
9	Chr#9	4	46
10	Chr#10	5	49
11	Chr#11	7	51
12	Chr#12	6	56
	Total	78	587

4.4.1 Parental polymorphism survey using SSR markers

A total of 587 SSR markers distributed evenly across all the twelve chromosomes were screened for parental polymorphism between Wazuhophek and ISM. Out of 587 SSR markers, 78 markers (13%) revealed polymorphism between the parents (Figure 4.5; Table 4.7). Highest numbers of eleven polymorphic markers were found from chromosome 1 followed by ten markers on chromosome 3 and nine markers on chromosome 7. The lowest value was recorded for chromosomes 5, 8 and 9. All the polymorphic markers were used for genotyping of RIL mapping population.

4.4.2 Phenotypic evaluation of RILs population for low soil P tolerance

4.4.2.1 Phenotypic evaluation of RILs population in low soil P and normal P soil condition

RILs under low soil P condition showed, susceptible, moderate tolerance and high tolerance response (Figure 4.6). Variations observed for physio-morphological traits in RILs population under low soil P and normal soil P condition for assessment of low soil P tolerance are summarized in Appendix A and Appendix B and described as;

The overall plant height of RILs under low soil P condition ranged from 40 cm to 96.33 cm with a mean value of 69.96 ± 1.10 cm. RIL no. 100 recorded a maximum height (96.33 cm) while the RIL no. 42 recorded the least height (40 cm). The overall mean plant height for parent Wazuhophek was 73 cm, while ISM had a height of 51 cm. The plant height under normal soil P condition varied from 77 (RIL no. 296) to 150 cm (RIL no. 213) with the mean of 104.72 ± 0.85 cm. The overall mean plant height under normal soil P for parent wazuhophek was 104 cm while ISM was 83 cm. The overall effective number of tillers/plant under low soil P condition ranged from 1 to 9 with a mean performance of 4.21 ± 0.16 . Maximum numbers of effective tillers were recorded in the RIL no. 296 and minimum numbers of effective tillers were recorded in the RIL no. 16, 19, 38 and 143. The overall mean of number of effective tillers for parent Wazuhophek and ISM were 8 and 2, respectively. The effective number of tillers/plant under normal soil P condition varied from 5.66 (RIL no. 87) to 18.33 (RIL no. 242)

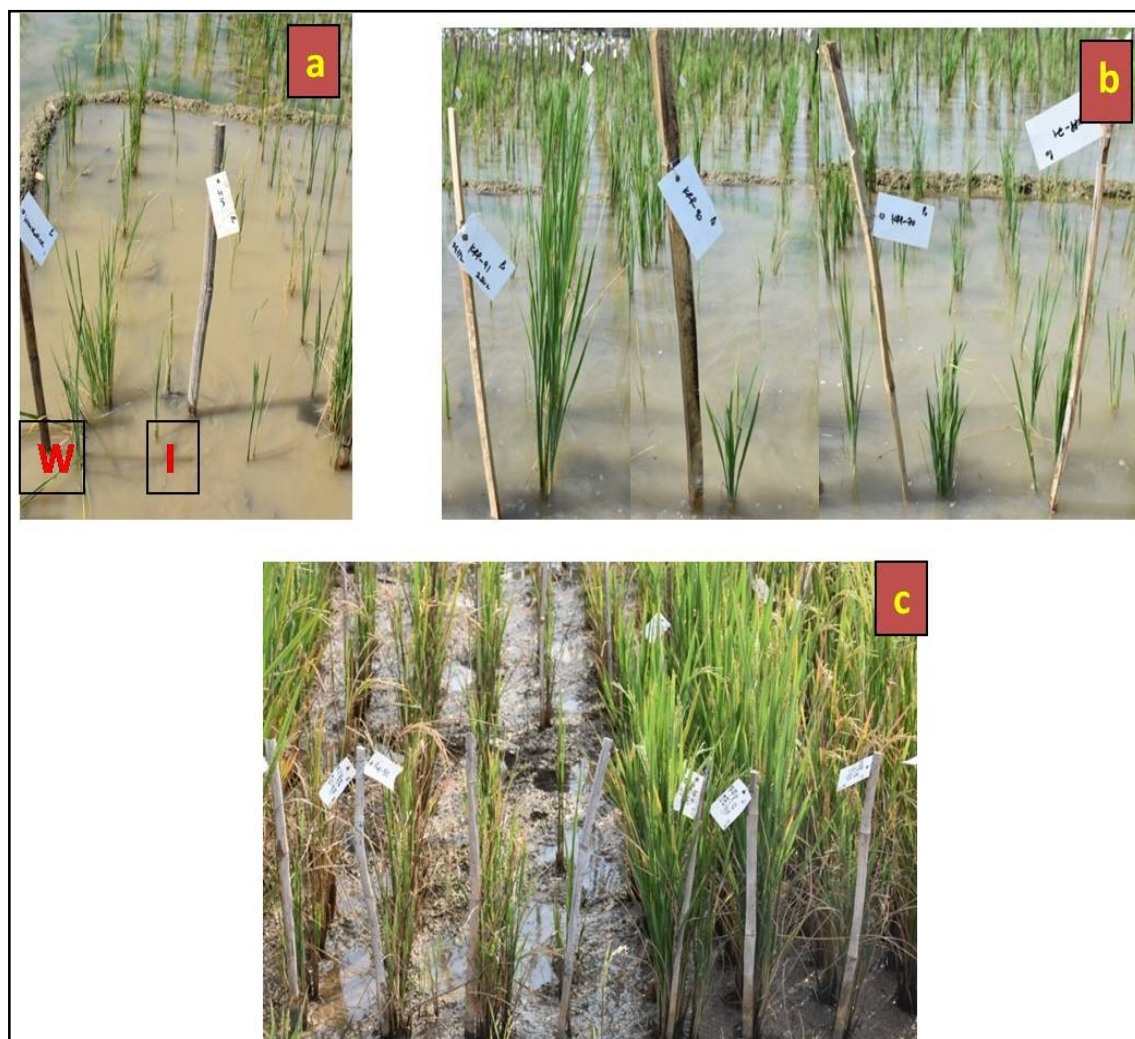


Figure 4.6: Screening of RILs population in low soil P plot at ICAR- IIRR, Hyderabad. a- Response of parents W (Wazuhophek), I (ISM) under low soil P plot at IIRR. b- Response of RILs under low soil P plot with variable degree of response for low soil P tolerance i.e from poor to good tolerance. c- Performance of individual RILs at flowering under low soil P

with the mean of 8.81 ± 0.18 . The overall mean effective number of tillers/plant under normal soil P for parent Wazuhophek was 12 while for ISM was 9.

The overall panicle length under low soil P condition ranged from 7 to 22 cm with a mean value of 14.29 ± 0.29 cm. Maximum panicle length was recorded in the RIL no. 295 and minimum panicle length were recorded in RIL nos. 27 and 42. The overall mean of panicle length for parent wazuhophek and ISM were 18 cm and 11 cm, respectively. The panicle length under normal soil P condition varied from 15.33 (ISM) to 27.66 (Wazuhophek) with the mean of 20.82 ± 0.17 cm. Maximum panicle length was recorded in the RIL no. 241 and minimum panicle length was recorded in the RIL no. 129. The overall shoot length under low soil P condition ranged from 37 to 89 cm with a mean performance of 61.25 ± 1.12 cm. Maximum shoot length was recorded in the RIL no.196 and minimum shoot length was recorded in the RIL no. 46. The overall mean of panicle length for parent wazuhophek and ISM were 68 cm and 48 cm, respectively. The shoot length under normal soil P condition varied from 70 (RIL no. 207) to 137 cm (RIL no. 213) with the mean of 98.43 ± 0.87 cm. The overall mean of shoot length for parent Wazuhophek and ISM were 97 cm and 80 cm, respectively. The overall root length under low soil P condition ranged from 14 to 32.5 cm with a mean performance of 23.23 ± 0.35 cm. Maximum root length was recorded in the RIL no.102 and minimum root length were recorded in the RIL no.39 and 217. The overall mean of panicle length for parent wazuhophek and ISM were 26 cm and 22 cm respectively. The root length under normal soil P condition varied from 18.5 cm (RIL no. 207) to 31.5 cm (RIL no. 164 and 242) with the mean of 25.17 ± 0.22 cm. The overall mean of root length in normal soil P condition for parent wazuhophek and ISM were 30 cm and 28 cm respectively.

The overall dry shoot weight under low soil P condition ranged from 0.9 to 13.35 g with a mean value of 6.30 ± 0.30 g. Maximum dry shoot weight was recorded in the RIL no. 296 and minimum dry shoot weight was recorded in the RIL no. 46. The overall mean of dry shoot weight for parent wazuhophek and ISM were 5.2 and 2 g, respectively. The overall mean of dry shoot weight for parent Wazuhophek and ISM were 30 and 10 g respectively. The dry shoot weight under normal soil P condition varied from 11.5 g (RIL no. 296) to 42.1 g (wazuhophek) with the mean of 20.32 ± 0.43 g. Maximum dry shoot weight was recorded in the RIL no.164 and minimum dry shoot

weight was recorded in the RIL no. 296. The overall mean of dry shoot weight for parent wazuhophek and ISM were 42.1 and 24.5, respectively.

The overall dry root weight under low soil P condition ranged from 0.1 to 6.45 g with a mean value of 1.21 ± 0.08 g. Maximum dry root weight was recorded in the RIL no.198 and minimum dry root weight were recorded in the RIL no.46 and 217. The overall mean of dry root weight for parent wazuhophek and ISM were 2.5 and 0.2 g, respectively. The dry root weight under normal soil P condition varied from 2.05g (RIL no. 207) to 9.95 g (RIL no. 6) with the mean of 4.52 ± 0.13 g. The mean dry root weight for parent wazuhophek and ISM were 7.9 and 3.5, respectively.

The overall root volume under low soil P condition ranged from 5 to 30 ml with a mean value of 15.39 ± 0.66 ml. Maximum root volume were recorded in the RIL no.182, 298, 299, 300, 301 and 302 and minimum root volume were recorded in the RIL no.16,25,46,131, 147,217 and 271. The overall mean of root volume for parent Wazuhophek and ISM were 30 and 10 ml, respectively. The root volume under normal soil P condition varied from 20 ml (RIL no. 204 and 207) to 140 ml (wazuhophek) with the mean of 69.47 ± 2.15 ml. Maximum root volume was recorded in the RIL no.65 and minimum root volume was recorded in the RIL no. 204 and 207. The overall mean of root volume for parent wazuhophek and ISM were 140 and 70 ml, respectively.

The average root shoot ratio under low soil P condition ranged from 0.05 to 0.76 with a mean value of 0.19 ± 0.008 g. RIL no. 198 showed a highest root shoot ratio while, the RIL no. 4 recorded the least yield per plant. The overall mean root shoot ratio for parent Wazuhophek was 0.49, while ISM was 0.10. The root shoot ratio under normal soil P condition varied from 0.09 (RIL no. 26) to 0.44 (RIL no. 42) with the mean of 0.22 ± 0.005 . The overall mean of root shoot ratio for parent Wazuhophek and ISM were 0.18 and 0.14 respectively.

The average grain yield per plant under low soil P condition ranged from 0.13 gm to 11.46 g with a mean value of 3.94 ± 0.28 g. RIL no. 203 showed a highest yield per plant while, the RIL no. 268 recorded the least yield per plant. The overall mean yield per plant for parent wazuhophek was 9 g, while ISM was 2.5 g. The grain yield per plant under normal soil P condition varied from 3.63 (RIL no. 215) to 20.2 (RIL no. 5) with a mean of 9.87 ± 0.03 . The overall mean of grain yield per plant for parent wazuhophek and ISM were 18.37 g and 16.3 g, respectively. The overall average 1000 seed weight under low soil P condition ranged from 1 gm to 2.72 g with a mean value of 1.66 ± 0.02 g. RIL no. 213 showed a highest 1000 seed weight, while the RIL no. 45

and 46 recorded the least yield per plant. The overall mean 1000 seed weight for parent wazuhophek was 2.21 g while for ISM it was 1.1 g. The 1000 seed weight under normal soil P condition varied from 7 g (RIL no. 260) to 25.5 g (RIL no. 11) with the mean of 18.27 ± 0.25 . The overall mean of 1000 seed weight for parent wazuhophek and ISM were 21.1 g and 12.2 g, respectively.

The overall average total biomass under low soil P condition ranged from 1.06 gm to 22.88 g with a mean value of 10.24 ± 0.51 g. Maximum total biomass was recorded in the RIL no.296 and minimum total biomass was recorded in the RIL no. 46. The overall mean of total biomass for parent wazuhophek and ISM were 14.2 and 4.5 g respectively. The total biomass under normal soil P condition varied from 17.98 (RIL no. 19) to 60.47 (wazuhophek) with the mean of 30.19 ± 0.62 g. Maximum total biomass was recorded in the RIL no.213 and minimum total biomass was recorded in the RIL no. 19. The overall mean of total biomass for parent wazuhophek and ISM were 60.47 g and 40.80 g, respectively.

The overall average days to 50 per cent flowering under low soil P condition ranged from 119 to 134 days with mean value of 126.68 ± 0.27 days. The highest and lowest days to fifty per cent flowering were recorded for the RIL no. 14 and 100, respectively. The parents wazuhophek and ISM took 134 and 122 days for fifty per cent flowering, respectively. The overall average P content in grains (mg g^{-1}) under low soil P condition ranged from 0.08 gm to 1.14 mg g^{-1} with a mean value of 0.45 ± 0.02 g. RIL no. 296 showed the highest P content in grains while, the RIL no. 42 recorded the least P content in grains. The overall mean P content in grains for parent wazuhophek was 0.5 g while for ISM, it was 0.09 g.

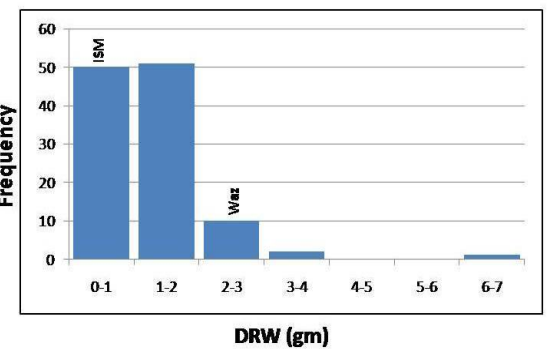
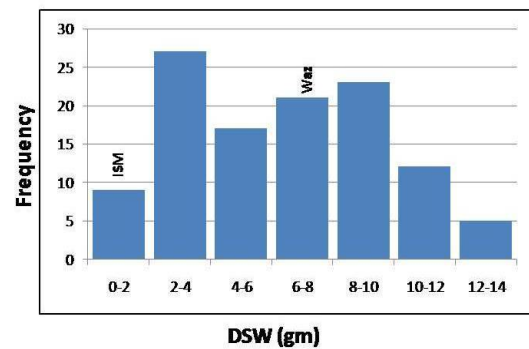
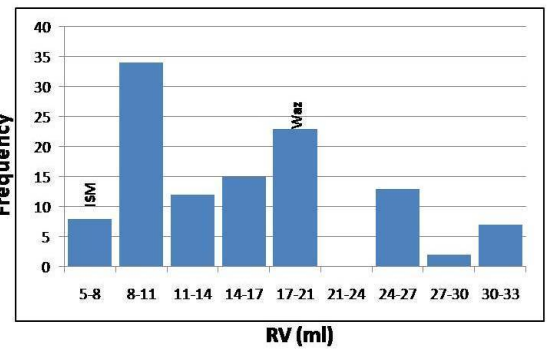
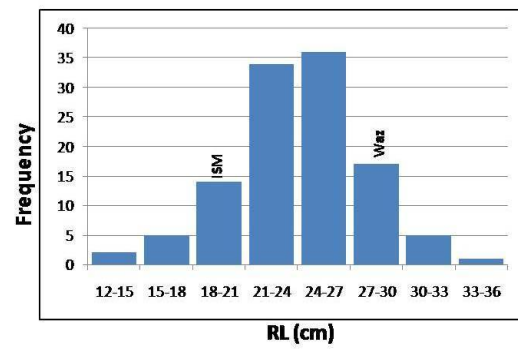
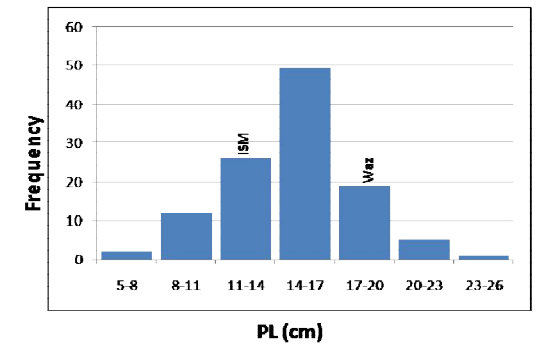
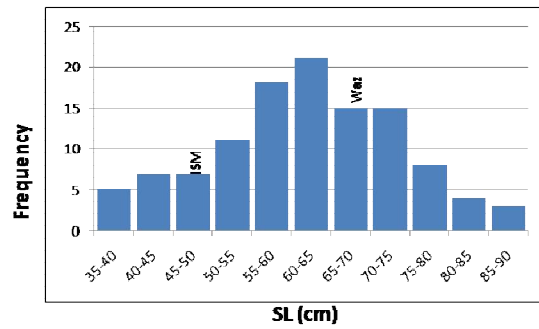
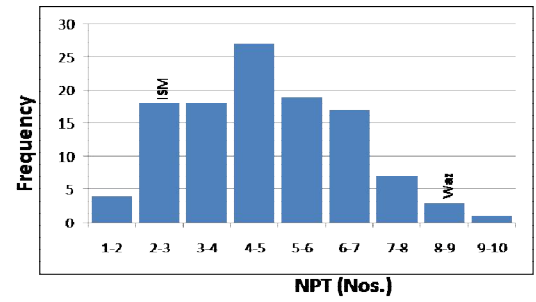
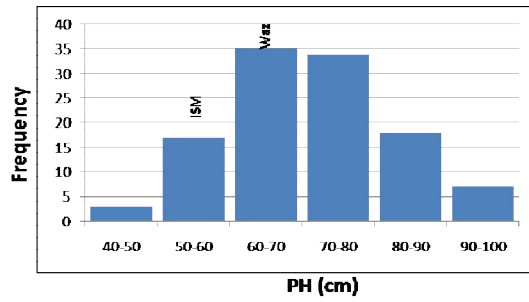
4.4.2.2 Frequency Distribution of low P tolerance related traits

Molecular mapping exercises can be aided by deployment of immortalized mapping populations such as recombinant inbred line (RIL) population derived from a divergent biparental cross (Qu *et al.*, 2008). Continuous distribution of phenotypic frequency of different traits with respect to screening in low soil P plot in the present study supports the quantitative inheritance of traits as expected for quantitative traits (figure 4.7). In bar charts Y axis (vertical axis) generally represents the frequency count, while the X- axis (horizontal axis) represents the variable being measured. Data with respect to overall mean, range, standard deviation, transgressive segregation, Kurtosis

and skewness of all the traits measured in 112 RILs under low soil P and normal soil P conditions are presented in Table 4.8 and Table 4.9 and explained as;

In the present study, it was found that the distribution of RILs for shoot length, panicle length, root length and days to 50 percent flowering were negatively skewed and the RILs were positively skewed with respect to plant height, number of effective tillers/plant, dry shoot weight, dry root weight, root volume, root shoot ratio, grain yield per plant, 1000 seed weight, total biomass and P content in grains morphological traits studied under low soil P condition. Maximum skewness was observed for root shoot ratio whereas, minimum was observed for days to fifty percent flowering. Under normal P soils negative skewness was observed for shoot length and 1000 seed grain weight and all other traits were positively skewed with maximum skewness for number of effective tillers/plant and minimum skewness for shoot length. Kurtosis, the fourth-degree statistic depicts degree of peakness of distribution of RILs. Near normal platykurtic distribution suggested the occurrence of high frequency of RILs with intermediate phenotype controlled by large number of genes. In the present study, it was observed during analysis of data except for dry shoot weight and root shoot ratio, all the morphological traits showed platykurtic distribution, since the value was below 3 for all the traits. Negatively skewed platykurtic distribution of RILs suggested the involvement of large number of genes with majority of them displaying duplicate epistasis while, positively skewed platykurtic distribution of RILs suggested the involvement of large number of genes with majority of them displaying complementary epistasis.

Normal distribution of histogram indicates the presence of additive effect with mid-parental value as the mean of population, while the presence of asymmetry in distribution or skewness in plot with transgressive segregation indicates epistatic interactions (Pooni *et al.* 1987). Screening of the RIL population under low soil P condition revealed that transgressive segregants are in the range of 5.26 % (root to shoot ratio) to 67.54 % (dry shoot weight) while under normal soil P condition it is 00 % (panicle length) to 95.61% (Grain yield per plant). The transgressive segregation in the RIL population is an indication of the presence of sufficient variability in the parental lines, polygenic nature of inheritance of target traits and occurrence of genetic recombination (Tiwari *et al.*, 2009; Falconer, 1989). A segregating population with heterozygosity can show effects of additive gene action with over-dominance and epistasis will affect superiority of transgressive individuals in successive generations (Cho *et al.* 2002; Kuczynska *et al.* 2007). However, the population used in this study



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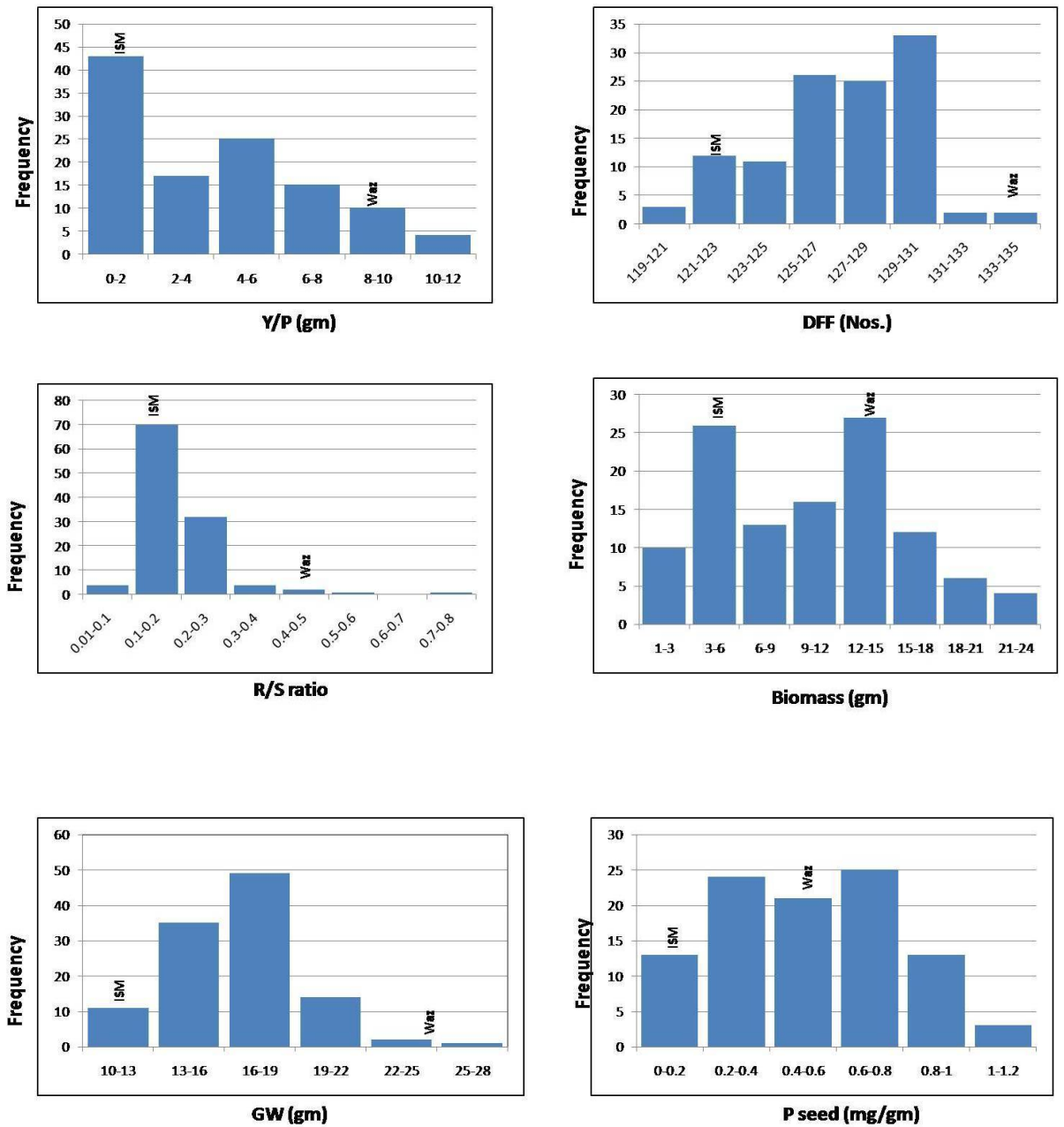


Figure 4.7: Phenotypic distribution of RILs for various low soil P related traits under low soil P field condition. Waz- Wazuhophek, ISM- improved samba Mahsuri, PH- plant height, NPT- number of productive tillers, SL- shoot length, PL- panicle length, RL- root length, RV- root volume, DSW- dry shoot weight, DRW- dry root weight, Y/P- yield pr plant, DFF- days to fifty percent flowering, R/S ratio- root to shoot ratio, GW- 1000 grain weight, P seed- P content in seed .

Table 4.8: Descriptive statistic data of yield and yield related traits obtained under low soil P condition

SI. No	Character	Wazuhophek	ISM	RIL's						
				Mean	SEd	Min	Max	% trans segregates	Skewness	Kurtosis
1	Plant height (cm)	73.0	51.0	69.9	11.8	40.0	96.33	42.1	0.02	-0.3
2	Shoot length (cm)	68.0	48.0	61.2	12.0	37.0	89.0	43.8	-0.02	-0.4
3	Number of productive tillers/plant	8.0	2.0	4.21	1.7	1.0	9.0	44.7	0.3	-0.3
4	Panicle length (cm)	18.0	11.0	14.2	3.1	7.0	22.6	20.1	-0.08	-0.1
5	Root length (cm)	26.0	22.0	23.2	3.7	14.0	32.5	50.0	-0.2	-0.04
6	Dry shoot weight (gm)	5.2	2.0	6.30	3.2	0.9	13.3	67.5	0.2	-0.9
7	Dry root weight (g)	2.5	0.2	1.21	0.8	0.1	6.4	8.7	2.4	10.9
8	Root volume (ml)	20.0	10.0	15.3	7.09	5.0	30.0	37.7	0.5	-0.7
9	Root shoot ratio	0.4	0.1	0.19	0.09	0.05	0.7	5.2	3.3	15.7
10	Grain yield per plant (g)	9.0	2.5	3.9	3.06	0.1	11.4	50.8	0.6	-0.6
11	1000 seed weight (g)	22.1	11.0	16.6	2.8	10.0	27.2	35.9	3	0.9
12	Total biomass (g)	14.2	4.5	10.2	5.5	1.06	22.8	42.1	0.2	-0.9
13	Days to 50 per cent flowering (Days)	134.0	122.0	126.6	2.9	119.0	134.0	7.8	-0.4	0.002
14	P content in grains (mg g ⁻¹)	0.5	0.09	0.45	0.3	0.08	1.1	56.5	0.14	-0.9

Table 4.9: Descriptive statistic of yield and yield related traits obtained under normal soil P condition

SI. No	Character	Wazuhophek	ISM	RIL's						
				Mean	SEd	Min	Max	% trans segregates	Skewness	Kurtosis
1	Plant height (cm)	104.0	83.0	104.7	9.1	77.0	150.0	52.6	0.9	5.9
2	Shoot length (cm)	97.0	80.0	98.4	9.3	70.0	137.0	59.6	-0.05	2.7
3	Number of productive tillers/plant	12.0	9.0	8.8	1.9	5.6	18.3	64.9	1.7	5.8
4	Panicle length (cm)	27.7	15.3	20.8	1.8	15.3	27.7	00.0	0.4	1.1
5	Root length (cm)	30.0	28.0	25.2	2.4	18.5	31.5	88.6	0.08	0.2
6	Dry shoot weight (gm)	42.1	24.5	20.3	4.6	11.5	42.1	84.2	1.1	3.5
7	Dry root weight (g)	7.9	3.5	4.5	1.48	2.05	9.9	25.4	1.2	2.2
8	Root volume (ml)	140.0	70.0	69.5	23.0	20.0	140.0	49.1	0.29	-0.1
9	Root shoot ratio	0.18	0.14	0.2	0.05	0.09	0.4	75.4	1.5	3.9
10	Grain yield per plant (g)	18.3	16.3	9.87	3.7	3.6	20.2	95.6	0.9	0.4
11	1000 seed weight (g)	21.1	12.1	18.3	2.7	7.0	25.5	14.9	-0.5	1.9
12	Total biomass (g)	60.4	40.8	30.2	6.7	17.9	60.4	92.1	1.27	3.4

were homozygous RILs and hence can be considered for molecular mapping studies involving quantitative traits.

4.4.2.3 Correlation analysis between traits under study

Effective selection among population can be done by studying correlation analysis among component traits. In the present study, correlation was determined to know the nature of relationship existing among 112 RILs and their parents for 14 quantitative characters under low soil P condition. Knowledge of the association between various agronomic characters is essential to conceptualize, identify, and develop ideal plant types through effective planning of recombination, and deployment of selection indices (Sreelakshmi *et al.*, 2010). The correlation matrix helps to select accessions with a desired combination of trait from the collection. A total of 91 pair wise combinations were formed among fourteen traits of which 65 combinations were found to be significant and positively correlated at 1% level and 5% level under low soil P condition (Table 4.10) and explained in detail below;

Under low soil P condition, plant height showed positive association with all the traits except days to fifty percent flowering and root to shoot ratio. Plant height showed maximum positive significant association with shoot length (0.90***) whereas low association was found with dry root weight (0.41***). Under normal soil P condition plant height showed significant positively association with shoot length (0.87***) and other six traits, while it showed non-significant association with number of productive tillers, panicle length, root length, and root to shoot ratio. Similar to the observation under low soil P condition, the plant height showed low positive association with dry root weight. Under low soil P condition shoot length, strong association was observed with total biomass (0.82***) and less association with dry root weight (0.48***). Shoot length showed negative association with days to fifty percent flowering and root to shoot ratio. Similar result were obtained under normal P condition, where shoot length showed higher positive association with total biomass (0.36***) and less positive association with dry root weight (0.21*). Shoot length showed non-significant association with number of productive tillers, panicle length, root length, and root to shoot ratio.

Number of effective tillers per plant (NPT) exhibited significant positive association with yield per plant (0.71***) while low association observed with root

Table 4.10: Values of the significant and positive correlation for yield and yield related traits in Improved Samba Mahsuri X Wazuhophek, RIL population under low soil P condition

Traits	Positive significant correlation	r value
Plant height	Shoot length***	0.90
	Effective number of tillers/plant***	0.48
	Panicle length***	0.78
	Root length***	0.50
	Root volume***	0.54
	Dry shoot weight***	0.66
	Dry root weight***	0.41
	Yield per plant***	0.64
	1000 seed weight***	0.49
	Total biomass***	0.74
	P content of seed***	0.50
Shoot length	Effective number of tillers/plant***	0.56
	Panicle length***	0.81
	Root length***	0.56
	Root volume***	0.61
	Dry shoot weight***	0.73
	Dry root weight***	0.48
	Yield per plant***	0.72
	1000 seed weight***	0.53
	Total biomass***	0.82
	P content of seed***	0.58
Number of productive tillers/plant	Panicle length***	0.66
	Root length***	0.36
	Root volume***	0.42
	Dry shoot weight***	0.45
	Dry root weight***	0.37
	Yield per plant***	0.71
	1000 seed weight***	0.37
	Total biomass***	0.65
	P content of seed***	0.49
Panicle length	Root length***	0.44
	Root volume***	0.43
	Dry shoot weight***	0.52
	Dry root weight***	0.38
	Yield per plant***	0.66
	1000 seed weight***	0.46
	Total biomass***	0.67
	P content of seed***	0.48
Root length	Root volume***	0.65

Traits	Positive significant correlation	r value
	Dry shoot weight***	0.62
	Dry root weight***	0.41
	Yield per plant***	0.40
	1000 seed weight**	0.29
	Total biomass***	0.58
	P content of seed***	0.38
Root volume	Dry shoot weight***	0.69
	Dry root weight***	0.48
	Yield per plant***	0.51
	1000 seed weight**	0.28
	Total biomass***	0.69
	P content of seed***	0.42
Dry shoot weight	Dry root weight***	0.51
	Yield per plant***	0.55
	1000 seed weight**	0.39
	Total biomass***	0.89
	P content of seed***	0.62
Dry root weight	Yield per plant***	0.39
	1000 seed weight***	0.32
	Total biomass***	0.52
	Root to shoot ratio***	0.55
	P content of seed***	0.30
Yield per plant	1000 seed weight***	0.57
	Total biomass***	0.87
	P content of seed***	0.61
1000 seed weight	Total biomass***	0.54
	P content of seed***	0.47
Total biomass	P content of seed***	0.70

Each significance level is associated to a symbol: p-values (0.001, 0.01, 0.05, 0.1) <=> symbols (***, **, *, ,)

length (0.36***). NPT showed non-significant association with root to shoot ratio under low soil P condition. Under normal soil P condition NPT showed higher positive association with total biomass (0.34***), while low association was observed with root length (0.19*) and non-significant association was observed with panicle length, dry root weight and 1000 seed weight and root to shoot ratio. Panicle length and Plant height showed high and significant association (0.78****) whereas panicle length showed low association with dry root weight (0.38***). Panicle length showed negative association with days to fifty percent flowering and non significant association with root to shoot ratio. Under normal soil P condition panicle length showed significant positive association with yield per plant, 1000 seed weight and total biomass while it showed non- significant association with remaining traits.

Root length showed maximum positive significant association with root volume (0.65****) and less association with 1000 seed weight (0.29***). Root length showed negative correlation with days to fifty percent flowering and root to shoot ratio. Under normal soil P condition Root length showed higher significant positive association with dry shoot weight (0.48****) and showed negative association with panicle length. Root volume exhibited significant positive association with dry shoot weight and total biomass (0.69****) and less association with 1000 seed weight (0.28***). Similar to results obtained under low soil P condition, root volume showed significant positive correlation with dry shoot weight (0.65****) and total biomass (0.53****) and less association with 1000 seed weight (0.19*) under normal soil P condition.

Dry shoot weight showed strong association with total biomass (0.89****) whereas less association with 1000 seed weight (0.29***) and negative association with days to fifty percent flowering and root to shoot ratio. Under normal soil P condition also, dry shoot weight showed strong positive association with total biomass (0.84****) and negative association with root to shoot ratio. Dry root weight exhibited significant positive association with root to shoot ratio (0.55****) and less association with 1000 seed weight (0.32***), while it showed negative association with days to fifty percent flowering. Data analysed from normal soil P condition showed significant association between dry root weight and root to shoot ratio (0.74****) followed by dry shoot weight (0.57****) and total biomass (0.47****). Under low soil P condition, grain yield showed strong association with total biomass (0.87****) while less association with dry root weight (0.39***). grain yield also showed good association with other traits such as

plant height (0.64***), shoot length (0.72***), number of productive tillers (0.71***), panicle length (0.66***), root length (0.40***), root volume (0.51***), dry shoot weight (0.55***), 100 seed grain weight (0.57***), P content in seed (0.61***). While grain yield showed negative and non significant association with days to fifty percent flowering and non significant association with root to shoot ratio. Similar result obtained under normal soil P condition where grain yield showed positive association with total biomass (0.74***).

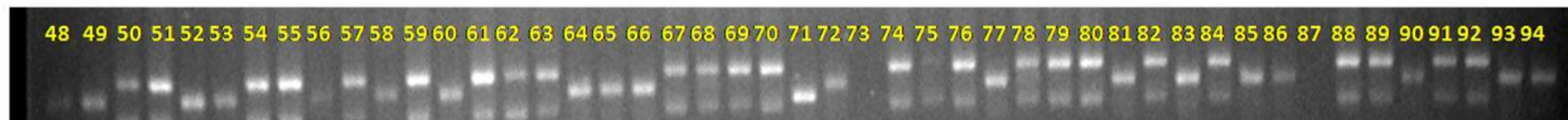
Grain yield also showed positive association with other traits such as plant height (0.25**), shoot length (0.23*), number of productive tillers (0.21*), panicle length (0.19*), dry shoot weight (0.26**). Whereas grain yield under normal P condition showed non-significant positive association with root length (0.11), root volume (0.14) and dry root weight (0.13) and negative association with root to shoot ratio (-0.04). A significant positive association was observed between 1000 seed weight and total biomass (0.54***), while 1000 seed weight showed negative association with days to fifty percent flowering and non-significant association with root to shoot ratio under low soil P condition. Except number of productive tillers, dry shoot weight, dry root weight and root to shoot ratio 1000 seed weight showed significant association with the remaining traits. Under both low soil P and normal soil P condition, root to shoot ratio showed significant association with dry root weight. Similarly total biomass also showed strong significant association with dry shoot weight under both the normal and low soil P condition. Under low soil P condition days to fifty percent flowering showed negative association with all the traits analysed in present study. Under low soil P condition P content in seed showed higher significant positive association with total biomass (0.70***), significant association with all other traits except with days to fifty percent flowering and root to shoot ratio which showed negative association.

The present study showed that most of the traits were positively associated with each other except for root to shoot ratio and days to fifty percent flowering which showed negative or non-significant association with most of the traits in study. This is accordance with previous studies (Fageria and Santos, 1998; Wissuwa *et al.*, 1998; Li *et al.*, 2009; Zhang *et al.*, 2017). Root dry weight and shoot dry weight showed relatively high correlations under low P condition whereas biomass showed relatively high association under both P sufficient and P deficiency conditions as reported by Chankaew *et al.* (2019). Similarly the number of productive tillers showed significant and positive association with root dry weight and shoot dry weight and biomass (Chankaew *et al.*, 2019). In the present study lines with more number of productive

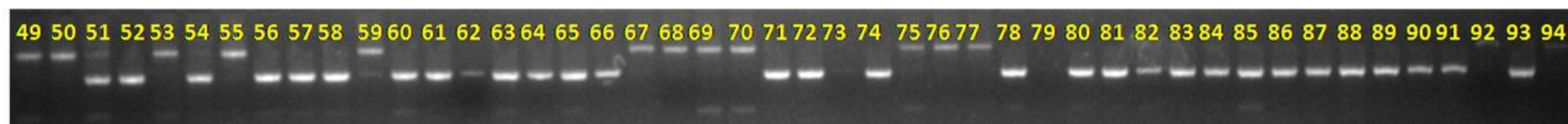
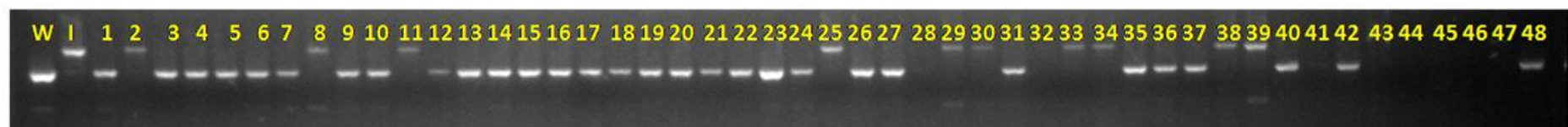
tillers were more tolerant to P deficiency as observed in earlier studies (Wissuwa and Ae, 2001; Aluwihare *et al.*, 2106; Chankaew *et al.*, 2019). Root shoot ratio recorded positive association with root dry weight, while negative association was observed with biomass and this result is in agreement with the result reported by Chankaew *et al.* (2019). Root length was significantly correlated with root volume, root dry weight and biomass in both P deficient and P sufficient condition, while plant height showed significant positive association with root volume, root dry weight and biomass in P deficient condition in this study. This is in congruence to the observations of Li *et al.* (2009) and Luo *et al.*, (2017). Grain yield per plant displayed significant and positive association with root length and thousand grain weight and similar observations were reported by Haider *et al.* (2012) in rice under drought stress condition. Considering the significant correlation among these traits, it is supposed that possible existence of QTL cluster(s) is probably the important genetic basis of association among related traits under phosphorus deficiency condition.

4.4.3 Genotyping of the RILs population using polymorphic SSR markers and linkage map construction

All the 78 parental polymorphic SSR markers (Table 4.7 and Figure 4.8) were used for genotyping the 112 RILs. Single plant analysis was carried out with the marker-genotype information, wherein each and every plant of the population was genotyped. The phenotypic and genotypic data of the 112 RILs were used for the construction of linkage map and QTL analysis. A linkage map was constructed using IciMapping version 4.0. Linkage groups were obtained using two-point analysis with a threshold LOD score of 2.5. The genetic length of each chromosome varied based on the number of markers in the linkage group. The linkage groups were assigned to different chromosomes based on presence of SSR markers in the respective groups in the RIL population of cross ISM/Wazuhophek. Results of linkage analysis are presented in Figure 4.9. Distribution of SSR loci on 12 chromosomes was as follows: Chromosome 1 (150.86 cM length), chromosome 2 (138.08 cM), chromosome 3 (201.59 cM), chromosome 4 (120.85 cM), chromosome 5 (81.09 cM), chromosome 6 (96.9 cM), chromosome 7 (244.98 cM), chromosome 8 (108.18 cM), chromosome 9 (86.08 cM), chromosome 10 (108.74 cM), chromosome 11 (106.14 cM) and chromosome 12 (189.56 cM). This covered 1633.05 cM on the all 12 chromosomes with an average difference of 20.93 cM between adjacent markers. The SSR linkage map developed was used in QTL mapping.



RM8-22554



SSR6-6.9

Figure 4.8: Segregation pattern of polymorphic SSR markers showing co-segregation with tolerance in the RIL mapping population: W- Wazuhophek, I- Improved Samba Mahsuri (ISM), 1 to 94- RIL genotypes

4.4.4 Mapping of QTL using RILs, screened for the low soil P tolerance

The association between the SSR markers with low soil P tolerance in the RILs population was studied using linkage analysis with QTL IciMapping V.4.1 software (Meng *et al.*, 2015). The genomic regions significantly influencing traits associated with low soil P tolerance were detected using Inclusive composite interval mapping (ICIM) method. In addition, mapping was also done using WinQTLCart version 2.5 software with the same data to crosscheck the results obtained. Results of QTL mapping are presented in Figure 4.9 a & b; Table 4.11; Table 4.12 and explained as;

4.4.4.1 QTLs detected on chromosome 1

With ICIM analysis, four QTLs were detected on chromosome 1. QTL obtained for dry shoot weight, which is denoted as qDSW1, was identified between the marker intervals of WR1.7- RM8105, with 3.24 LOD score, contributing 14.11 % to the phenotype variance. A QTL for total biomass denoted as qBiomass1, was identified between the markers WR1.7- RM8105, with 3.10 LOD score, accounting 15.44 % to the phenotypic variation. Another QTL for root to shoot ratio named as qr1 was identified between the markers WR1.7- RM8105 with 3.10 LOD score accounting 15.44 % to the phenotypic variation for low soil P tolerance. A QTL for P content in seed named qSP1, was identified between the markers WR1.7- RM8105, with 2.75 LOD score and accounting 10.51 % to the phenotypic variation. When QTL analysis was performed with WinQTLCart version 2.5 using CIM, three QTLs viz., qDSW1, qBiomass1, qSP1 were detected, on chromosome 1, between the markers WR1.7- RM8105. The QTL qDSW1 was identified with LOD score 3.29 and the phenotypic variation was 48.55%, qBiomass1 was identified with LOD score 6.82 and phenotypic variation of 47.38%, while qSP1 was identified with LOD score 2.79 and phenotypic variation of 51%.

Wang *et al.* (2014), conducted a two-year field experiment under low and normal P conditions using a recombinant inbred population and investigated three yield and eight Phosphorus use efficiency related traits including grain P use efficiency (PUE). Of the 36 QTLs and 24 epistatic interactions, seven QTLs and three epistatic interactions affected by environment and four QTLs (qPHI1 and qPHI2 for PHI, qPUEg2 for PUEg, and qPTE8 for PTE) with strong effects were found to be non-dependent on environment. Nine QTLs for different traits including yield traits and phosphorus use efficiency were identified on chromosome 1 in the present study. We

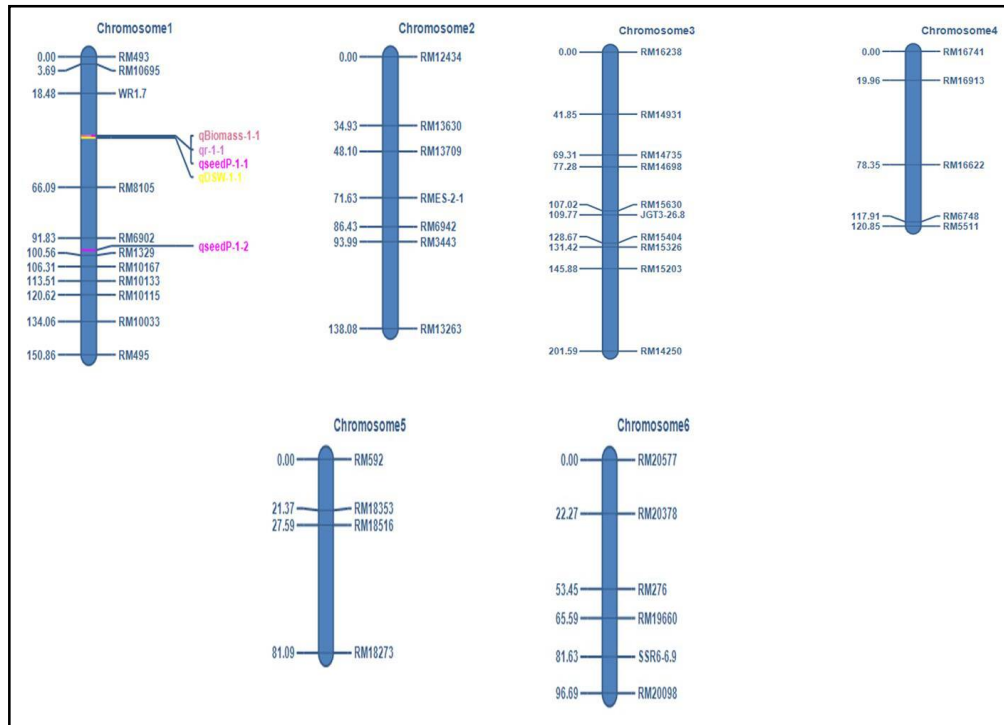


Table 4.11: QTL(s) detected for low soil P tolerance in RIL population of Improved Samba Mahsuri X Wazuhophek

SI. no	Trait	Chr No.	Associated markers	LOD	PVE(%)	Add	Position (cM)	Position (Mb)	Size (Mb)
1	Plant Height (PH)	8	RM22554(L)-RM8005(R)	4.22	15.28	5.83	11	5.5-1.01	4.49
2	Shoot length (SL)	8	RM22554(L)-RM8005(R)	3.32	17.25	4.49	10	5.5-1.01	4.49
3	Number of productive tillers (NPT)	8	RM22554(L)-RM8005(R)	6.02	21.84	0.96	12	5.5-1.01	4.49
4	Panicle length (PL)	8	RM22554(L)-RM8005(R)	5.97	20.23	1.79	10	5.5-1.01	4.49
5	Dry shoot weight (DSW)	1	WR 1.7(L)-RM8105(R)	3.24	14.11	2.34	41	1.7-10.9	9.2
6	Dry shoot weight (DSW)	8	RM22554(L)-RM8005(R)	3.83	5.81	1.5	10	5.5-1.01	4.49
7	Root volume (RV)	7	RM5847(L)-RM22031 (R)	3.05	14.33	-3.4	231	23.59-26.3	2.71
8	Root volume (RV)	8	RM22554(L)-RM8005(R)	4.68	14.24	3.39	14	5.5-1.01	4.49
9	Yield (SY)	8	RM22554(L)-RM8005(R)	5.5	18.5	1.76	10	5.5-1.01	4.49
10	Biomass	8	RM22554(L)-RM8005(R)	5.89	11.42	3.21	11	5.5-1.01	4.49
11	Biomass	1	WR 1.7(L)-RM8105(R)	3.1	15.44	3.73	40	1.7-10.9	9.2
12	Root/shoot ratio	8	RM22554(L)-RM8005(R)	5.89	11.42	3.21	11	5.5-1.01	4.49
13	Root/shoot ratio	1	WR 1.7(L)-RM8105(R)	3.1	15.44	3.73	40	1.7-10.9	9.2
14	P content of Seed	1	WR 1.7(L)-RM8105(R)	2.75	10.51	0.23	40	1.7-10.9	9.2
15	P content of Seed	7	RM21521(L)-RM21103(R)	2.87	10.01	-0.22	87	15.9-4.59	11.31
16	P content of Seed	8	RM22554(L)-RM8005(R)	2.75	2.25	3.21	9	5.5-1.01	4.49

Table 4.12: Comparative table of QTL analysis for low soil P tolerance with RILs population using ICIMqtl mapping and WInQTL cartographer mapping software

QTL Mapping using RILs, phenotyped for low P tolerance at IIRR, Rajendranagar, Hyderabad										
		Results obtained with ICIM					Results obtained with Cartographer			
QTL	Chr.no	Flanking Markers	LOD	Additive Effect	Dominance Effect	Phenotypic variance (%)	LOD	Additive Effect	Dominance Effect	Phenotypic variance (%)
<i>qPH</i>	8	RM22554(L)- RM8005(R)	4.22	5.83	0	15.28	7.4	7.95	0	41.55
<i>qSL</i>	8	RM22554(L)- RM8005(R)	3.32	4.49	0	17.25	6.16	6.99	0	32.73
<i>qNPT</i>	8	RM22554(L)- RM8005(R)	6.02	0.96	0	21.84	3.06	0.74	0	17.85
<i>qPL</i>	8	RM22554(L)- RM8005(R)	5.97	1.79	0	20.23	7.11	1.8	0	32.11
<i>qDSW</i>	1	WR 1.7(L)- RM8105(R)	3.24	2.34	0	14.11	3.29	2.26	0	48.55
<i>qDSW</i>	8	RM22554(L)- RM8005(R)	3.83	1.5	0	5.81	4.17	1.5	0	21.52
<i>qRV</i>	7	RM5847(L)- RM22031 (R)	3.05	-3.4	0	14.33	3.18	-4.33	0	37.24
<i>qRV</i>	8	RM22554(L)- RM8005(R)	4.68	3.39	0	14.24	5.18	4.07	0	32.49
<i>qYield</i>	8	RM22554(L)- RM8005(R)	5.5	5.5	0	18.5	5.75	1.9	0	36.84
<i>qBiomass</i>	1	RM22554(L)- RM8005(R)	5.89	5.89	0	11.42	6.82	3.83	0	47.38
<i>qBiomass</i>	8	WR 1.7(L)- RM8105(R)	3.1	3.1	0	15.44	7.07	3.41	0	36.49

have identified QTLs for biomass and P content of seed which also identified in the study conducted by Wang *et al.* (2014), but its map location is different. In another study, Luo *et al.* (2017), used a population of backcross recombinant inbred lines (BILs) for QTL mapping for phosphorus deficiency tolerance and identified QTLs on chromosome 1 for relative root dry weight, relative shoot dry weight, relative root length, relative shoot fresh weight and relative total biomass dry weight. Chao *et al.* (2015), has studied genetic basis of low phosphorus tolerance (LPT) in backcross introgression lines (BILs) under three treatments and locations and tested 11 quantitative traits. They reported identification of QTLs on chromosome 1 for traits like relative total dry weight, relative shoot dry weight relative total dry weight, relative root length, panicle number of plant, straw P use efficiency, P translocation efficiency, spikelet fertility and grain yield. The QTLs on chromosome 1 have also been identified for phosphorus deficiency related traits like relative total dry weight and P-deficiency induced activity of acid phosphatase (Li *et al.*, 2009; Hu *et al.*, 2001)

4.4.4.2 QTLs detected on chromosome 7

On chromosome 7, two QTLs were detected through ICIM analysis. QTL obtained for root volume, which is denoted as qRV7, was identified between the marker interval RM5847-RM22031 with 3.05 LOD score, contributing to 14.33 % to the phenotype variance. The QTL for P content in seed, denoted as qSP7, was identified between the markers RM21521-RM21103 with 2.87 LOD score, accounting 10.01 % to the phenotypic variation. When QTL analysis was performed with WinQTLCart version 2.5 using CIM, both the QTLs viz., qRV7 and qSP7 were detected on chromosome 7 between the markers RM5847-RM22031 and RM21521-RM21103, respectively. The qRV7 QTL was identified with LOD score 3.18, and phenotypic variation of the QTL was 37.24% and qSP7 identified with LOD score 2.97 and phenotypic variation of 51%. Previous studies related to QTL mapping for low soil P tolerance in rice crop have identified QTLs on chromosome 7 that contributed for phosphorus deficiency condition (Luo *et al.*, 2017; Zhang *et al.*, 2017; Chao *et al.*, 2015; Ping *et al.*, 2008).

Luo *et al.* (2017) have identified a significant QTL for relative root dry weight for low phosphorus tolerance on chromosome 7 which contributed for 10.39% of phenotypic variation with the LOD score of 2.89. An additional QTL was identified for relative shoot dry weight with the phenotypic variation of 14.6%. Zhang *et al.* (2017)

carried out study with the goal of identifying genomic regions (quantitative trait loci) that influence tolerance to Pi deficiency (TPDE) in hybrid rice (*Oryza sativa* L.), using recombinant inbred lines (RILs). Twenty-one additive QTLs were detected and jointly explained between 10–49% of the trait variance, while two QTLs for relative root length with the phenotypic variation of 24.92% and 10.49% were identified on chromosome 7 under P deficiency condition. A Study carried out by Chao *et al.* (2015) identified QTLs for low phosphorus tolerance on chromosome 7 for the traits, relative root dry weight, relative shoot dry weight, relative root length and thousand grain weight. Ping *et al.* (2008) analyzed a doubled haploid (DH) population of rice under phosphorus deficiency to carry out QTL mapping and identified three QTLs on chromosome 7 for, total number of panicle per plant, grain number per panicle and grain yield, among which QTL region for total number of panicle per plant found to be tightly linked showed significant phenotypic variation.

4.4.4.3 QTLs detected on chromosome 8

Through ICIM analysis, a total ten QTLs were detected on chromosome 8 for different traits related to low soil P tolerance. QTL obtained for plant height denoted as qPH8, was identified between the marker interval RM22554 - RM8005 with 4.22 LOD score, contributing 15.28 % to the phenotype variance. The QTL for shoot length denoted as qSL8, was identified between the markers RM22554 - RM8005, with 3.32 LOD score, accounting 17.25 % to the phenotypic variation for the low soil P tolerance. qNPT8, the QTL for number of productive tillers (NPT) was identified between the markers RM22554 - RM8005, with 6.02 LOD score, accounting 21.84 % to the phenotypic variation. For the trait panicle length, the QTL, qPL8 was identified between the markers RM22554 - RM8005, with 5.97 LOD score, accounting 20.23 % to the phenotypic variation. The QTL for dry shoot weight, DSW8 was identified between the markers RM22554-RM8005 with 3.24 LOD score accounting 14.11 % to the phenotypic variation. The QTL for root volume, denoted as qRV8, was identified between the markers RM22554 - RM8005, with 4.68 LOD score, accounting 14.24 % to the phenotypic variation. A QTL has identified for grain yield per plant, denoted as qYield8, was identified to be flanked by the markers RM22554 - RM8005, with 5.50 LOD score and accounting 18.50 % to the phenotypic variation. The QTL for total biomass denoted as qBiomass8 was identified between the markers RM22554 - RM8005 with 5.89 LOD score, accounting 11.42 % to the phenotypic variation. A QTL for root to shoot ratio, denoted as qr8, was identified between the markers RM22554 -

RM8005 with 5.89 LOD score, accounting 11.42 % to the phenotypic variation. A QTL was obtained for P content of seed (denoted as qSP8) was identified between the markers RM22554 - RM8005, with 2.75 LOD score, accounting 2.75 % to the phenotypic variation.

When QTL analysis was performed with WinQTLCart version 2.5 using CIM, eight common QTLs were identified, viz., qPH8, qSL8, qNPT8, qPL8, qDSW8, qRV8, qYield8 and qBiomass8. Interestingly these QTLs were identical to those identified through ICIM analysis. All the eight QTLs identified were located between the marker interval RM22554 - RM8005 and they are qPH8, qSL8, qNPT8, qPL8, qDSW8 qRV8, qYield8 and qBiomass8 with LOD scores, 7.40, 6.16, 3.06, 7.11, 4.17, 5.18, 5.75 and 7.07, respectively and contributing to phenotypic variation of 41.45, 31.73, 17.85, 32.11, 32.11, 32.49 , 36.84 and 36.49 %, respectively.

Luo *et al.* (2017), identified a QTL for relative root dry weight with LOD score of 2.53 and it contributed for 6.13 % of phenotypic variation. Wang *et al.* (2014), used the high density SNP map in rice for identification of QTL for phosphorus use efficiency. Two QTLs were identified on chromosome 8 for P translocation and P translocation efficiency that contributing for 5% and 7.5 % of phenotypic variation, respectively. As per literature study, only the above mentioned QTLs have been identified on chromosome 8. It is pertinent to note that many of the QTLs identified on Chromosome 8 are novel and hence will be useful in breeding.

4.4.5 *In silico* identification of the genes present within QTL intervals

The annotated Nipponbare rice genome sequence (2009) map-set available at Gramene website (www.gramene.org) used to find out the physical position of the major QTLs on different rice chromosomes based on the position of flanking markers of QTL genomic region. The genomic region underlying selected QTLs were searched for putative candidate genes by utilizing rice database RAP-DB. *In silico* analysis of identified QTL region found to contain genes which are involved in the low soil P tolerance response. Such information can be used to identify probable target genes for expression studies such as real-time PCR. Many researchers have used *in silico* approach to shortlist candidate genes from mapped QTLs for different biotic stresses like sheath blight, gall midge (Yadav *et al.*, 2015; Yasala *et al.*, 2012) and abiotic stress related traits like drought (Pradeepa *et al.*, 2012; Wang *et al.*, 2005b) and quality

related traits like phenolic, grain protein content in rice and other crops (Verdu *et al.*, 2014; Chandel *et al.*, 2011; Descalsota *et al.*, 2018).

4.4.5.1 *In silico* identification of the genes present within QTL intervals on chromosome 1

Four QTLs (qr1, qDSW1, qBiomass1, qSP1) identified on chromosome 1 at genomic region of 1.7 to 10.9 and flanked by markers WR1.7- RM8105 were considered for *in silico* analysis, which span across 9.2 Mb region on chromosome 1 or 2.41 % of the rice genome (373.24 Mb). For *in silico* analysis, we have considered some extra region from both of the sides *i.e.* 1 Mb-11.5Mb (10.5 Mb). A total of 1339 genes are predicted in the region spanning 1 to 11.5 Mb region of chromosome 1 and these genes are involved in various biological processes, biotic and abiotic stress response with some of the encoding proteins with unknown function. Among the 1339 genes, 14 genes appear to be putatively related to phosphorus uptake or utilization and their details are summarized in table 4.13a.

Out of 14 genes, four genes were related to auxin responsive activity which contribute for root growth and cell growth which indirectly involved in low soil P tolerance (Giri *et al.*, 2018). Other genes related to acid phosphatase activity and other enzymes that help in phosphorus translocation and phosphorus transport also identified in the genomic region spanning the QTL. Various researchers demonstrated that root exudates of phosphatase activity and the up regulation of genes related to phosphatase in roots increases in response to P deficiency in several plants including rice (Tadano *et al.*, 1993; Teng *et al.*, 2013).

4.4.5.2 *In silico* identification of the genes present within QTL intervals on chromosome 7

A genomic region of size 23 to 27 Mb on chromosome 7 encompassing a single QTL for root volume between marker interval of RM5847- RM22031, was used for *in silico* analysis to identify genes present in this region. A total of 712 genes predicted in the analyzed region of chromosome 7, out of which only 12 genes possibly related to phosphorus uptake or utilization were identified. Their details are summarized in table 4.13b. Similarly, another QTL for P content in seed on chromosome 7 was identified between the genomic region of 4 to 16 Mb and marker interval of RM21521-RM21103 was also subjected for *in silico* analysis. In this region, 16 genes of a total of 570 genes

Table 4.13a: List of annotated genes present within QTL intervals on chromosome 1 , related to phosphorus utilization and uptake

Locus ID	Position (bp) (start-end)	No. of Exons	Description (RAP-DB annotation)
Os01t0127200-01	1,497,241-1,500,606	4	Multicopper oxidase, Maintenance of Pi homeostasis
Os01t0138600-01	2,063,615-2,065,995	1	phosphotransferase system, PEP-utilising enzyme, N-terminal domain containing protein
Os01t0139600-01	2,097,192-2,100,692	9	Similar to Lipid phosphate phosphatase 2 (EC 3.1.3.-) (AtLPP2) (Phosphatidic acid phosphatase 2) (AtPAP2) (Prenyl diphosphate phosphatase)
Os01t0165000-01	3,356,383-3,358,426	2	Transcription factor, Dehydration and salt stress tolerance
Os01t0169800-01	3,577,473-3,581,859	5	Tryptophan aminotransferase, Indole-3-acetic acid (IAA) biosynthesis
Os01t0172100-01	3,711,212-3,713,653	9	Similar to Triose phosphate/phosphate translocator, non-green plastid, chloroplast precursor (CTPT)
Os01t0178500-02	4,073,916-4,076,438	5	A member of rice Aux/IAA family, Cross-talk of auxin and brassinosteroid signaling pathways, Plant morphogenesis
Os01t0191200-02	4,872,158-4,873,594	2	Similar to Acid phosphatase
Os01t0191700-01	4,905,790-4,909,119	1	Similar to Pyrophosphate-fructose-6-phosphate 1-phosphotransferase-like protein (Pyrophosphate-dependent phosphofructo-1-kinase-like protein)
Os01t0231000-03	7,249,868-7,253,703	5	Similar to Auxin-responsive protein (Aux/IAA) (Fragment).
Os01t0236300-01	7,547,017-7,551,842	14	Similar to Auxin response factor 18
Os01t0239000-02	7,686,901-7,693,781	6	Similar to Phosphate starvation regulator protein (Regulatory protein of P- starvation acclimation response Psr1)
Os01t0239200-01	7,718,270-7,722,217	12	Similar to Phosphate translocator (Fragment)
Os01t0259600-01	8,701,299-8,708,612	1	Similar to phosphoadenosine phosphosulfate (PAPS) reductase family protein

related to phosphorus uptake or utilization and their details are summarized in table 4.13c. The annotated genes identified in the present study on chromosome 7, for reported QTLs for root volume and P content of seed includes genes related to auxin responsive activity as well as for various transcription factors involved in stress response activity.

Wang *et al.* (2009a), showed that OsSPX1 acts via a negative feedback loop to optimize growth under phosphate-limited conditions. A gene coding for protein with SPX domain, which could be involved in negative regulation of phosphate starvation signaling was also indentified in present study. WD40 repeat-containing proteins play a key role in protein-protein or protein-DNA interactions by acting as scaffolding molecules and promoting protein activity under abiotic stresses with plant cell wall formation and other cellular activity which helps to increase tolerance level of plant (Kong *et al.*, 2015; Guerriero *et al.*, 2015). In the present study, the QTL, qSP7 on chromosome 7 showed presence of a gene coding for WD40 protein, which may be contributing for regulation of the plasma membrane localization of phosphate transporters, phosphate uptake and phosphate translocation. LAX3 expression is regulated by SHR transcription factor, and LAX3 contributes to adventitious roots formation which play important role under a biotic stress condition to show the tolerance (Della Rovere *et al.*, 2015). In present study, a gene coding for SHR transcription factors has been identified in QTL region of qRV7 on chromosome.

4.4.5.3 *In silico* identification of the genes present within QTL intervals on chromosome 8

A total of 10 QTLs are identified on chromosome 8 between the genomic regions of 1.01- 5.5 Mb and this appears to be the most important region associated with low P tolerance in the present study. A genomic region of size 0 to 6 Mb on chromosome 8 encompassing 10 QTLs for various p deficiency related traits was used for *in silico* analysis to identify gene content in this region. Of the total 538 genes predicted in the region of interest, eight genes were found to be related to phosphorus uptake or utilization and the details are summarized in table 4.13d. Subfamily of auxin-induced SMALL AUXIN UP-RNA (SAUR) genes are known to promote cell expansion and

Table 4.13b: List of annotated genes present within QTL intervals on chromosome 7, (23 to 27 mb genomic region) related to phosphorus utilization and uptake

Locus ID	Position (bp) (start-end)	No. of Exons	Description
Os07t0569700-01	22,930,745-22,933,283	2	Zinc finger AN1 and C2H2 domain-containing stress-associated protein, Stress response, Regulation of the expression of stress-associated genes
Os07t0572800-01	23,146,051-23,149,345	7	WNK (With No Lysine) kinase, Ser/thr protein kinase family member, Abiotic stress tolerance, Internal circadian rhythm
Os07t0576100-00	23,314,373-23,319,116	2	Similar to indole-3-acetic acid amido synthetase.
Os07t0576500-01	23,325,199..23,327,227	1	Similar to Auxin-responsive GH3-8 protein
Os07t0581366-00	23,540,795..23,543,383	1	Zinc finger, C2H2 domain containing protein
Os07t0586900-01	23,868,417..23,871,087	1	SHR transcription factor, Formation of root endodermis and leaf stomata, Regulation of the number of cortex cell layers in the root
Os07t0592600-01	24,149,718-24,152,074	2	Indole-3-acetic acid (IAA)-amido synthetase, Auxin signaling and defense signaling in a pathogen-nonspecific manner
Os07t0609000-01	25,049,566-25,050,625	1	Stress responsive alpha-beta barrel domain containing protein
Os07t0614700-02	25,326,762..25,327,995	1	SPX domain (SYG1, Pho81 and XPR1)-containing protein, Negative regulation of phosphate starvation signaling
Os07t0618500-02	25,524,505-25,530,261	7	Similar to Dihydropterin pyrophosphokinase /dihydropteroate synthase precursor (EC 2.5.1.15)
Os07t0623200-02	25,790,418-25,792,526	4	ATPase, P-type, K/Mg/Cd/Cu/Zn/Na/Ca/Na/H-transporter domain containing protein
Os07t0624600-01	25,867,212-25,871,232	11	Similar to Trehalose-6-phosphate phosphatase

Table 4.13c: List of annotated genes present within QTL intervals on chromosome 7, (4 to 16.5 mb genomic region) related to phosphorus utilization and uptake

Locus ID	Position Start – End (bp)	No. of Exons	Description (RAP-DB annotation)
Os07t0176600-01	4049784-4054583	15	Serine/threonine protein kinase, Never-in-mitosis A (NIMA)-related kinase, Pollen germination
Os07t0176900-02	4068003-4070134	1	Similar to ribose-5-phosphate isomerase.
Os07t0179400-01	4185365-4187968	6	Dephospho-CoA kinase domain containing protein.
Os07t0182400-01	4355211-4356572	3	AUX/IAA protein family protein.
Os07t0185000-01	4499107-4501911	5	Similar to MAP kinase (Ser/Thr kinase).
Os07t0186200-01	4593094-4595607	1	S-domain receptor-like kinase, Abiotic stress tolerance
Os07t0187400-00	4663426-4667478	1	ATPase, AAA-type, core domain containing protein.
Os07t0187700-01	4682480-4687646	1	WD40 protein, Regulation of the plasma membrane localization of phosphate transporters, Phosphate uptake and translocation
Os07t0190000-01	4821207-4824665	4	Similar to 1-deoxy-D-xylulose 5-phosphate synthase 2 precursor.
Os07t0191000-01	4890273-4892660	11	Inositol monophosphatase family protein.
Os07t0191200-01	4914656-4920788	21	Plasma membrane H ⁺ ATPase (EC 3.6.3.6).
Os07t0191600-04	4943798-4946602	2	Half-size ABC transporter, Mycorrhizal arbuscule formation
Os07t0207700-01	5805378-5811087	1	Protein containing CTD phosphatase domain, Seed shattering
Os07t0211400-01	6052570-6060083	1	Similar to Kinase associated protein phosphatase.
Os07t0438800-01	14753753-14759288	2	MYB-CC family protein, Pi-starvation signaling
Os07t0438800-02	14753759-14757053	6	Similar to Phosphate starvation response regulator-like protein.

provide a mechanistic link between auxin and plasma membrane H⁺-ATPases (PM H⁺-ATPases), which take part in auxin-mediated control of plant cell expansion under stress condition (Spartz *et al.*, 2014). In the present study, two genes encoding for SAUR like protein in the genomic interval spanning the QTL has been identified. This may be contributing for auxin response under low P stress condition and helps in growth of root related parameters. A gene encoding *Zea mays* L. protein phosphatase 2A regulatory subunit A, designated *ZmPP2AA1*, was induced in roots by low Pi availability and data reveal the biological function of *ZmPP2AA1*, provide insights into a linkage between auxin and low Pi responses, and drive new strategies for the efficient utilization of Pi by maize (Wang *et al.*, 2016). Garbers *et al.*(1996), reported that protein phosphatase 2A play an important role in the regulation of auxin transport in Arabidopsis which ultimately influence the auxin mediate growth response under various stress condition. In present study, a gene encoding a similar to protein phosphatase 2A B' regulatory subunit was identified. Various reports have shown that protein belonging to the family of plant serine/threonine protein kinases contribute to abiotic stress signaling (Mao *et al.*, 2010; Franza *et al.*, 2011). In this study, a gene encoding protein containing Serine/threonine protein kinase related domain has been identified in the genomic region spanned by the QTL. In addition to the above mentioned genes, genes encoding protein containing Myb, Zinc finger domain and acid phosphatase or vanadium-dependent haloperoxidase related family proteins have also been identified in the region of interest. These genes have been reported to be involved in abiotic or phosphorus deficiency tolerance response (Winter and Moore, 2009; Zang *et al.*, 2016). A detailed analysis and validation of these candidate genes will facilitate understanding their possible role at molecular level with respect to low soil P tolerance.

This study documented that the genomic region on chromosome 8 between the marker interval of RM22554 and RM8005 showed epistatic interaction with the region on chromosome 11, between the marker interval of RM26784 and RM27096 with the 31.82% of phenotypic variation contribution. For the trait, panicle length epistatic interaction observed between genomic region of chromosome 7, between the marker interval of RM5720 and RM21521 with genomic region on chromosome 11, between the marker interval of RM26550 and RM26784 with the 20.31% of phenotypic variation. Another epistatic interaction observed for trait, root volume between the

Table 4.13d: List of annotated genes present within QTL intervals on chromosome 8, related to phosphorus utilization and uptake

Locus ID	Position (bp) (start-end)	No. of Exons	Description
Os08t0118500-00	1,039,566-1,040,187	1	Similar to Auxin-induced SAUR-like protein
Os08t0118800-00	1,045,709-1,046,017	1	Similar to SAUR31 - auxin-responsive SAUR family member
Os08t0122000-01	1,205,886-1,209,749	3	Similar to Protein phosphatase 2A B' regulatory subunit
Os08t0127500-01	1,578,268-1,580,227	5	Acid phosphatase/vanadium-dependent haloperoxidase related family protein
Os08t0135100-01	1,994,522-1,998,857	8	Similar to Phosphate/phosphoenolpyruvate translocator protein-like
Os08t0138700-01	2,159,517-2,162,918	7	Serine/threonine protein kinase-related domain containing protein
Os08t0144000-01	2,433,858-2,436,741	3	Myb, DNA-binding domain containing protein
Os08t0151700-01	2,972,601-2,976,414	9	Zinc finger, RING/FYVE/PHD-type domain containing protein

genomic region on chromosome 2, present between the marker interval of RM13709 and RMES-2-1, and genomic region on chromosome 8, present between the marker interval of RM8005 and SSR-8-19 with phenotypic variation value of 27.83%. A fourth significant epistatic interaction observed for biomass and root to shoot ratio trait on chromosome 8, with the marker interval of RM8005 and SSR-8-19 and genomic region on chromosome 12 between the marker interval of RM28346 and RM5479 with contribution of phenotypic variation of 24.90% (Fig.4.10, Table. 4.14).

In present study, screening of germplasm lines for low P tolerance have identified the rice line, IC216831 with significantly higher level of tolerance to low soil P, in comparison to the sensitive genotypes. During genotypic screening with *Pup1* associated markers, IC216831 was found completely devoid of *Pup1*, alleles. It can be speculated that it may possess novel genomic loci associated to low soil P tolerance and hence could serve as a novel source for low soil P tolerance after dissection of the genetic basis of tolerance and molecular mapping of the tolerance loci.

In case of second objective of study a new source of low soil P tolerance Wazuhophek, was used as a donor parent with a low P sensitive cultivar, ISM to produce recombinant inbred lines (RILs) for quantitative trait locus (QTL) analysis. Correlation analysis study of 14 traits among RILs showed that most of the traits have significant association with each other, whereas, root to shoot ratio and days to fifty percent flowering showed non-significant or negative association other traits in study. QTL analysis with 78, polymorphic simple sequence repeat (SSR) markers and composite interval mapping, identified four major main-effect QTLs (contributing >15 % of phenotypic variation) on chromosomes 8. Another 10 minor QTLs (contributing < 15 % of phenotypic variation) distributed on chromosome 1, 7 and 8. Based on the QTLs loci detected in this study, we could find that most of the QTLs including for yield and other agro-morphological traits were present on chromosome 8, between the marker interval of RM22554 and RM8005 and may be consider as hot spot for QTLs. Four QTLs were related to number of productive tillers, Panicle length and yield per plant and shoot length in the present study contributed for higher phenotypic variation, which suggested that these four QTLs Viz., qNPT8, qPL8, qSL8 and qYiled8 are critical for responding to the signal of P-deficiency stress. So, they are logical selection targets for breeding rice with higher productivity in low phosphorus soils. Studies have shown that

Table 4.14: Epistatic interaction observed for various traits studied for low soil P tolerance

Trait Name	Chr 1 ^a	Position1 (cM) ^c	Flanking markers Chr 1 ^a	Chr 2 ^a	Position2 (cM) ^d	Flanking markers Chr 2 ^a	LOD	PVE(%)	Add ^a	Add ^b	Add byAdd
NPT	Chr 8	0	RM22554-RM8005	Chr 11	75	RM26784 - RM27096	5.67	31.83	0.4996	- 0.0493	1.24
PL	Chr 7	10	RM-5720-RM21521	Chr 11	15	RM26550 - RM26784	5.29	20.32	0.3764	0.4673	-1.95
RV	Chr 2	65	RM13709-RMES-2-1	Chr 8	40	RM8005-SSR-8-19	5.84	27.84	- 1.2722	2.1145	-4.88
Biomass	Chr 8	40	RM8005-SSR-8-19	Chr 12	30	RM28346 -RM5479	5.62	24.91	1.2826	- 0.3760	-4.37
root shoot ratio	Chr 8	40	RM8005-SSR-8-19	Chr 12	30	RM28346	5.62	24.91	1.2826	-0.376	-4.37

Note:- NPT- number of productive tillers, PL- Panicle length, RV- root volume, PVE%- phenotypic variation, Add- additive, LOD- logarithm of the odds, Add by Add- Additive by additive effect, Chr1^a- the first chromosome and QTL chromosomal interval, Chr2^a- the second chromosome and QTL chromosomal interval, Add^a- additive effect of the first QTL involve in interaction, Add^b- additive effect of the second QTL involve in interaction, c- position of first chromosome , d- position of second chromosome.

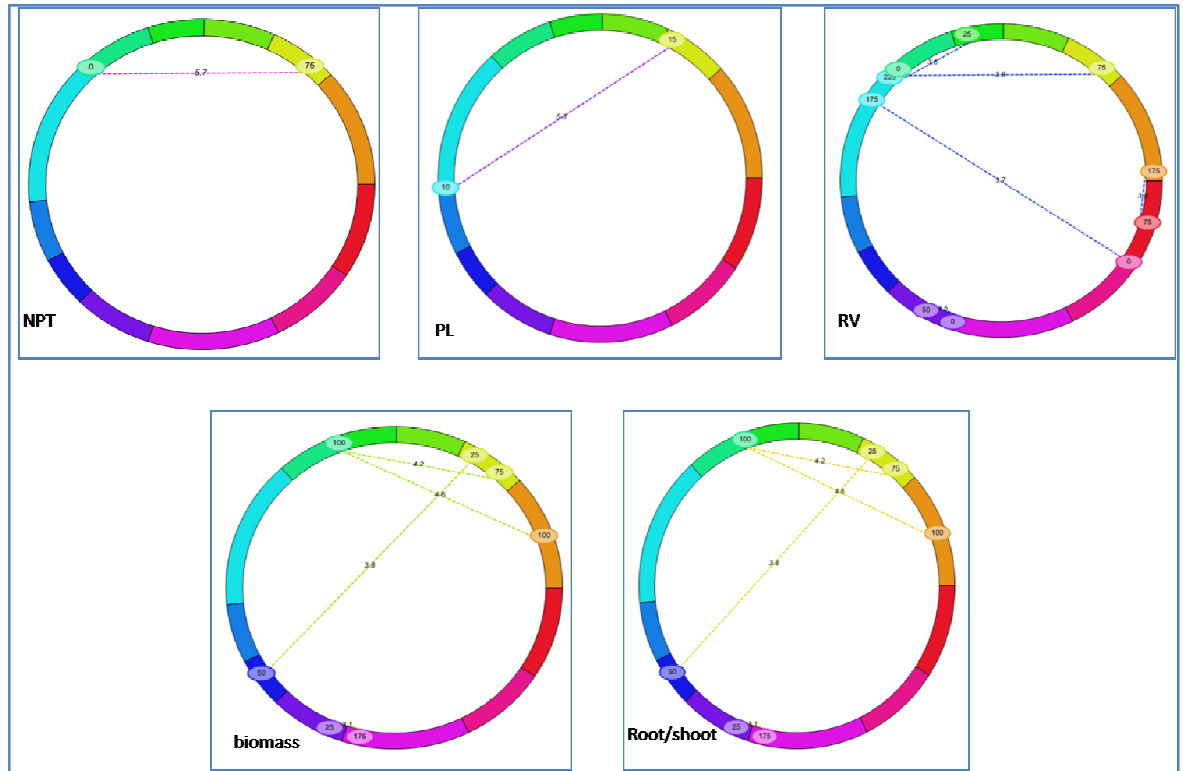


Figure 4.10: Epistatic interaction observed for various traits studied for low soil P tolerance. NPT- number of productive tillers, PL- panicle length, RV- root volume, root/shoot- root to shoot ratio

plants that are more efficient in P acquisition presented higher number of productive tillers, long panicle length and shoot length and more yield per plant. Some overlapped intervals could explain larger phenotypic variation with positive additive effect, which suggested that there were multiple major QTLs related to low-phosphorus tolerance. In brief, among all QTL detected in present study none QTL found to be have the overlapping interval with reported genes/QTL, whereas, additional genetic studies are needed to correctly understand the role of the QTLs identified in our study for low soil P tolerance. RILs further need to be analyzed to fine map the QTL genomic regions and determine precisely the effect of each QTL for increased tolerance to low soil P in terms of yield under low P condition. Evaluation of these traits in other populations with different parents would be useful to determine the consistency of QTL detection for the same trait in various backgrounds or to be able to detect QTLs with larger effects for these traits. All QTLs detected in this study should be validated in field experiments which will enrich the genetic resources and accelerate rice breeding against soil P deficiency.

SUMMARY AND CONCLUSION

Chapter V

SUMMARY AND CONCLUSION

As an essential macro-element for all living cells, phosphorus (P) is indispensable in all agricultural production systems. India has the largest rice growing area in the world and reports indicate that a majority of the rice growing soils are low in available P, causing significant yield losses. Farmers are applying more and more P fertilizers to get good crop yields, thus increasing their cost of production. It is important to develop phosphorus-efficient crops due to limited availability of phosphorus from soil and natural phosphorus reserves. P fertilizers are primarily sourced from non-renewable phosphate rock reserves, which are limited to a few countries and are predicted to be exhausted in near future. In case of rice, assessment of genetic and phenotypic variability for low soil P tolerance, showed reasonable variation in the ability to acquire and utilize P under P-deficient condition. With the rapid developments in molecular biology, QTL analysis has become a key tool for analyzing the genetic basis of complex traits like P-deficiency tolerance. A major quantitative trait locus (QTL) for phosphorus-deficiency tolerance, *Pup1*, was identified in the traditional aus-type rice variety Kasalath and successfully used in marker assisted backcross breeding. Further *Pup1*-specific protein kinase gene, which named phosphorus-starvation tolerance 1 (*PSTOL1*) contributing for root growth under low soil P condition was identified. In this regard, rice landraces can be considered as good source for identification and mining of novel low soil P tolerance QTLs / genes. Rice landraces, particularly from North-East India are genetically diverse since North-East India is one of the centres of origin for rice. North-East India is also one of the hotspots for the low soil P tolerance and this is the reason, rice landraces from this region evolved many novel sources of low soil P tolerance genes and QTLs. Such landraces are worthy candidates for a detailed examination of the low soil P tolerance genes and QTLs. Wazuhophek is one of the unique landraces of North-East India, showing good level of resistance to low soil P condition and an earlier study carried out at ICAR-IIRR, Hyderabad established that it is devoid of *Pup1* and hence may be a novel source of tolerance (Swamy *et al.*, 2019).

Keeping this information in view, the present research work entitled “Molecular mapping of genetic loci associated with tolerance to low soil phosphorus in rice (*Oryza sativa* L.)” was designed to identify novel source for low P tolerance and to decipher the

genetic basis of low soil P tolerance in Wazuhophek an already identified novel source of low soil P tolerance.

The results obtained from the present study are summarized as follows

- ✓ Analysis of soil in the low P plot of ICAR-IIRR revealed the presence of very low level of available P ($< 2 \text{ kg ha}^{-1}$).
- ✓ Analysis of variance revealed the presence of significant difference between the germplasm lines for days to fifty percent flowering, number of productive tillers, flag leaf length, root length, panicle length, shoot length, dry shoot weight, root volume, biomass, grain yield per plant under low soil P condition but significant difference was not found for flag leaf width.
- ✓ Estimation of GCV and PCV revealed the presence of high genetic variability for root length, plant height, number of productive tillers and flag leaf length under low soil P condition. Heritability estimates were high for all the traits. High genetic advance as percent mean for all the traits except panicle length under low P condition revealed the presence of ample scope for improvement of these traits under low P condition through selection.
- ✓ Stress indices like stress tolerance index, tolerance index, yield reduction ratio, yield index, stress susceptibility index, yield stability index and percent yield reductions were worked out based on the yield under normal condition and yield under the low soil P condition. Cluster analysis using these indices grouped the genotypes into three groups, viz., highly tolerant, tolerant and sensitive varieties. Eighteen entries including tolerant check Swarna were grouped under tolerant category. Remaining germplasm lines along with the sensitive checks were grouped under the sensitive category.
- ✓ Genotyping of the 56 germplasm lines using *Pup1* specific markers indicated the presence of *Pup1* region (complete or partial) in more than eighty percent of the genotypes. The *Pup1* was completely absent in low soil P sensitive checks Improved Samba Mahsuri (ISM) and nine germplasm lines namely IC216610, IC216731, IC216767, IC216831, IC216903, IC217339, IC21678, IC217567 and IC217684. The complete *Pup1* region was found in 21 germplasm lines and also in the tolerant check, Swarna.
- ✓ Based on the phenotypic performance of the germplasm lines under low soil P condition and genotypic analysis with *Pup1* associated DNA markers, a novel

source for low soil P tolerance, i.e. IC216831 was identified. It showed tolerance to low soil P.

- ✓ Phenotypic correlation coefficient analysis of recombinant inbred lines (RILs) derived from the cross Wazuhophek/Improved Samba Mahsuri revealed a positive association between almost all traits except days to fifty percent flowering and roots to shoot ratio, which showed negative or non significant association with other traits in our study.
- ✓ A total 78 markers of 587 SSR markers distributed evenly across all the twelve chromosomes were screened for parental polymorphism between Wazuhophek and ISM revealed polymorphism between the parents.
- ✓ A linkage map was constructed using RIL population with genotyping data of 78 polymorphic markers, which covered a total of 1633.05 cM covering all the 12 chromosomes and with 20.93 cM average distance between adjacent markers.
- ✓ Using 112 RILs and 78 parental polymorphic markers, a total of 16 QTLs were identified for various physio-morphological traits associated with low soil P tolerance (plant height, number of productive tillers, panicle length, grain yield/plant, shoot length, dry shoot weight, root volume, biomass, root to shoot ratio and P content of seed) and they are distributed on chromosome viz., 1, 7 and 8.
- ✓ Total four QTLs were identified on chromosome 1, contributing significantly to dry shoot weight (qDSW1), total biomass (qBiomass1), root to shoot ratio (qr1) and P content of seed (qSP1), which are located between the marker interval of WR1.7-RM8105, explaining 14.11%, 15.44%, 15.44% and 10.51% of phenotypic variation, respectively.
- ✓ A total of two QTLs were identified on chromosome 7, contributing to root volume (qRV7) and P content of seed (qSP7), located between the marker interval of RM5847-RM22031 and RM21521-RM21103, contributing to phenotypic variation of 14.33% and 10.01%, respectively.
- ✓ Ten QTLs contributing towards plant height (qPH8), shoot length (qSL8), number of productive tillers (qNPT8), panicle length (qPL8), dry shoot weight (qDSW8), root volume (qRV8), yield per plant (qYiled8), biomass (qBiomass8), root/shoot ratio (qr8) and P content of seed (qSP8) were identified on chromosome 8, between the marker interval of RM22554-RM8005, contributing to phenotypic variation in the range of 2.25 to 21.84%.

- ✓ *In silico* analysis, identified a total 1339 genes in the 1 to 10 mb region of chromosome 1, out of which 14 genes were found to be putatively related to phosphorus uptake and / or utilization, including auxin responsive genes and genes involved in maintenance of Pi homeostasis.
- ✓ A total of 712 genes were predicted in the 23 to 27 mb region of chromosome 7, and among them 12 genes were found to be putatively related to phosphorus uptake and / or utilization, including auxin responsive genes and transcription factors involved in regulation of stress related genes.
- ✓ Similarly, another QTL for P content in seed on chromosome 7, was identified between the genomic region of 4 to 16 Mb and marker interval of RM21521-RM21103, was also subjected for *in silico* analysis. In this region, out of total of 570 genes predicted, 16 were found to be possibly related to phosphorus uptake or utilization.
- ✓ *In silico* analysis showed that a total 538 genes are present in the 0 to 5.5 mb region of chromosome 8, of which eight genes were found to be putatively related to phosphorus uptake and / or utilization, including include genes related to auxin signalling, phosphatase activity and zinc finger transcription factor that plays role in P starvation condition.
- ✓ Complex epistatic interactions were observed between for trait grain yield per plant, days to fifty percent flowering, dry shoot weight and P content of seed.

Breeding crops tolerance to low soil P stress is an important research area for rice breeders. Development of crop plants with high P use efficiency and tolerance to low soil P will greatly reduce the P application in the form of fertilizers and helpful in reducing the cost of cultivation, thereby help in increasing the farmer's income. Further the novel, low P tolerant germplasm line, IC216831 identified in the present study, could serve as donor for low P tolerance after dissection of the QTLs/genes associated with tolerance. While analyzing the RIL mapping population derived from the cross Wazuhophek / Improved Samba Mahsuri, all of the traits showed continuous distribution and transgressive segregation, reflecting quantitative inheritance nature and thus, further QTL analysis was necessary to define the actual genes that are involved in low P soil tolerance. With the largest phenotypic variation of the detected QTL, the trait number of productive tillers followed by panicle length, seemed to be the most effective traits for evaluating low soil P tolerance and will be used for phenotypic scanning in the future fine mapping of low soil P tolerance genes. Further the low P tolerant and

bacterial blight resistant RILs derived from the cross Wazuhophek / Improved Samba Mahsuri identified in the present study, possessing one or more major QTLs associated with tolerance will enrich the genetic resources and accelerate rice breeding programmes, after the validation of the QTLs in alternate mapping populations.

LITERATURE CITED

LITERATURE CITED

- Abebe, T., Alamerew, S and Tulu, L. 2017. Genetic variability, heritability and genetic advance for yield and its related traits in rainfed lowland rice (*Oryza sativa* L.) genotypes at Fogera and Pawe, Ethiopia. *Advances in Crop Science and Technology*. 5:2. DOI:10.4172/2329-8863.1000272.
- Ahlawat, I.P.S and Saraf, C.S. 1981. Response of pigeon pea to plant density and phosphorus fertilizer under dryland conditions. *Journal of Agricultural Sciences* (Cambridge). 97: 119-124.
- Ahmad S., Yaseen, M and Saboor, A. 2000. Genetic variation for phosphorus use in rice at two levels of soil applied phosphorus. *Pakistan Journal of Biological Sciences*. 3(8):1274-1276.
- Akinrinde, E.A and Gaizer, T. 2006. Differences in the performance and phosphorus-use efficiency of some tropical rice (*Oryza sativa* L.) varieties. *Pakistan Journal of Nutrition*. 5(3): 206-211.
- Alhasnawi, A.N., Kadhimi, A.A., Isahak, A., Ashraf, M.F., Doni, F., Mohamad, A., Yusoff, W.M.W.M and Zain, C.R.C.M. 2015. Application of Inter Simple Sequence Repeat (ISSR) for Detecting Genetic Analysis in Rice (*Oryza sativa* L.). *Journal of pure and applied microbiology*. 9(2): 1091-1101.
- Allard, R.W. (1960). *Principles of Plant Breeding*. Wiley, New York.
- Al- Turki and Basahi, M.A. 2015. Assessment of ISSR based molecular genetic diversity of Hassawi rice in Saudi Arabia. *Saudi Journal of Biological Sciences*. 22. 591–599.
- Aluwihare, C., Ishan, M., Chamikara, M.D.M., Weebadde, C.K., Sirisena, D.N., Samarasinghe, W.L.G and Sooriyapathirana, S.D.S.S. 2016. Characterization and selection of phosphorus deficiency tolerant rice genotypes in Sri Lanka. *Rice Science*. 23(4):184-195.
- Aluwihare, Y.C., Chamikara, M.D.M., Dissanayake, D.R.R.P., Karannagoda, N.N.H., Sirisena, D.N., Samarasinghe, W.L.G., Rajapakse, S and Sooriyapathirana, S.D.S.S. 2015. Validation of K46, a *Pup1*-linked marker, using a selection of Sri Lankan rice (*Oryza sativa* L.) germplasm for marker assisted selection towards phosphorous deficiency tolerance. *Ceylon Journal of Science*. 44 (2): 45-54.

- Ameenul, M., Shalini, T., Govintharaj, P., Manonmani, S and Robin, S. 2016. Assessment of parental genetic variability in rice. *International Journal of Agriculture Sciences*. 8(54): 2917-2919.
- Anila, M., Mahadeva Swamy, H.K., Kale, R.R., Bhadana, V.P., Anantha, M.S., Brajendra., Hajira, S.K., Balachiranjeevi, C.H., Ayyappa Dass,M., Bhaskar, S., Dilip, T., Pranathi, K., Kousik, M. B. V. N., Harika,G., Swapnil, K., Chaitra, K., LaxmiPrasanna, B., Punniakotti, E., Sinha, P., Rekha, G., Abhilash Kumar,V., Balachandran,S.M., Madhav, M.S., Giri, A., Viraktamath, B.C and Sundaram, R. M. 2018. Breeding lines of the Indian mega-rice variety, MTU 1010, possessing protein kinase *OsPSTOL (Pup1)*, show better root system architecture and higher yield in soils with low phosphorus. *Molecular Breeding*.38:147.
- Anila, M., Mahadevaswamy, H.K., Kale, R.R, Bhadana, V.P, Brajendra, P., Hajira, S.K., Pranathi, K., Balachiranjeevi, CH., Praveen, M., Abhilash Kumar, V., Bhaskar, S., Dhanalaxmi, D., Harika, G., Dilipkumar, T., Kousik, MBVN., Yugender, A., Punniakotti, E., Laxmi Prasanna, B., Swapnil, K., Rekha, G., Balachandran, S.M., Neeraja, C.N., Madhav, M.S., Surekha, K., Laha, G.S., Anantha, M.S, Fiyaz, A., Suneetha, K., Gireesh, C., Viraktamath, B.C., Ravindra Babu, V and Sundaram, R.M. 2017. Marker Assisted Introgression of *Pup1*, A Major QTL Associated with Tolerance to Low Soil Phosphorus into Elite Rice Varieties. *ICAR-IIRR News Letter* –15 (1):26-29.
- Anis, G.B., Zhang, Y., Wang, H., Li, Z., Wu, W., Sun, L., Riaz, A., Cao, L and Cheng, S.2018. Genomic Regions Analysis of seedling root traits and their regulation in responses to phosphorus deficiency tolerance in CSSLs population of elite super hybrid rice. *Int.J.Mol. Sci*.19:1460-1474.
- Ashraf, A., El-Mohsen, A., Abd El-Shafi, M.A., Gheith, E.M.S and Suleiman H.S. 2015.Using Different Statistical Procedures for Evaluating Drought Tolerance Indices of Bread Wheat genotypes. *Advance in Agriculture and Biology*. 3(1): 19-30.
- Assuero, S.G., Mollier, A and Pellerin S. 2004. The decrease in growth of phosphorus deficient maize leaves is related to a lower cell production. *Plant, Cell and Environment*. 27:887-895.
- Barber, S.A. 1995. Soil Nutrient Bioavailability a mechanistic approach. 2nd ed. John Wiley and Sons Inc., New York

- Bates, T.R and Lynch, J.P. 1996. Stimulation of root hair elongation in *Arabidopsis thaliana* by low phosphorus availability. *Plant, Cell and Environment*.19:529-538.
- Bentur, J.S and Viraktamath, B.C. 2008. Rice plant hoppers strike back. A report on second international rice conference on rice plant hoppers held at IRRI, Philippines during 23-25 June 2008. *Current Science*. 95: 4.
- Blair, M.W., Panaud, O and McCouch, S.R. 1999. Inter-simple sequences repeat (ISSR) amplification for analysis of microsatellite motif frequency and fingerprinting in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics*.98 (5): **780–792**.
- Botstein, D., White, R. L., Skolnick, M and Davis, R.W. 1980. Construction of genetic linkage map in man using restriction length polymorphisms. *Am J Hum Genet*. 32:314-331.
- Bousslama, M and Schapaugh, W.T. 1984. Stress tolerance in soybean. Part 1: evaluation of three screening techniques for heat and drought tolerance. *Crop Science*.24: 933- 937.
- Brears, R.C. 2015.The circular economy and the water-food nexus. *Future Food J Food Agric Soc*.3(1):53-59.
- Buresh, R.J., Smithson, P.C and Hellums, D.T. 1997. Building soil phosphorus capital in Africa. In: Buresh RJ, Sanchez PA, Calhoun F (eds) Replenishing soil fertility in Africa. SSSA SSSA special publication No. 51:111–150.
- Burton, G.W and De-Vane, E.W. 1953. Estimating heritability in all fescue (*Festuca aurandinacea*) from replicated clonal material. *Agronomy Journal*. 45: 478-481.
- Cakmak, I. 2002. Plant nutrition research: priorities to meet human needs for food in sustainable ways. *Plant Soil*. 247:3–24.
- Cancellier, E.L., Brandao, D.R., Silva, J., Santos, M.M and Fidelis, R.R. 2012. Phosphorus use efficiency of upland rice cultivars on Cerrado soil. *Ambience*. 8(2): 307-318.
- Chandel, G., Dubey, M., Samual, P and Meena, R. 2011.*In silico* mining and characterization of novel SSRs and candidate genes within QTLs controlling grain protein contents using MPSS signatures and micro array analysis in rice (*Oryza sativa* L.). *Plants Omics Journal*. 4(3):126-135.

- Chankaew, S., Monkham, T., Pinta, W., Sanitchon, J., Kaewpradit, W and Srinives, P. 2019. Screening Tolerance to Phosphorus Deficiency and Validation of *Phosphorus Uptake 1 (Pup1)* Gene- Linked Markers in Thai Indigenous Upland Rice Germplasm. *Agronomy*. 9:81; doi:10.3390/agronomy902008
- Chao, Z., Jie, R., Qin, Z.X., Song, D.Z., Jing, Z., Chao, W., Wei, Z.J., Joseph, C.A., Qiang, Z and Yun-Long, P. 2015. Genetic dissection of low phosphorus tolerance related traits using selected introgression lines in rice. *Rice Science*. 22(6):264–274.
- Cheema, K.K., Grewal, N.K., Vikal, Y., Sharma, R., Lore, J.S., Das, A., Bhatia, D., Mahajan, R., Gupta, V., Bharaj, T.S and Singh, K. 2008. A novel bacterial blight resistance gene from *Oryza nivara* mapped to 38 kb region on chromosome 4 and transferred to *Oryza sativa* L. *Genetical Research*. 90:1-11.
- Chen, D., Wang, G.L and Ronald, P.C. 1997. Location of the rice blast resistance locus *Pi5(t)* in Moroberekan by AFLP bulk segregant analysis. *Rice Genetics News Letter*. 14:95-97.
- Chen, K., Zhan, Q., Xing, Q., Wang, Z., Jin, D., He, D and Wang, B. 2003. Tagging and mapping of rice sheath blight resistant gene. *Theoretical and Applied Genetics*. 106: 293-297.
- Chen, H., Xie, W., He, H., Yu, H., Chen, W., Li, J., Yu, R., Yao, Y., Zhang, W., He, Y., *et al.* 2014. A high-density SNP genotyping array for rice biology and molecular breeding. *Mol. Plant*. 7: 541–553.
- Chiangmai and Yodmingkhwan. 2011. Competition of root and shoot growth between cultivated rice (*Oryza sativa* L.) and common wild rice (*Oryza rufipogon* Griff.) grown under different phosphorus levels. *Songklanakarin Journal of Science and Technology*. 33(6): 685-692.
- Chin, J.H., Gamuyao, R., Dalid, C., Bustamam, M., Prasetyono, J., Moeljopawiro, S., Wissuwa, M and Heuer, S. 2011. Developing rice with high yield under phosphorus deficiency: Pup1 sequence to application1. *Plant Physiology*. 156(3): 1202–1216.
- Chin, J.H., Lu, X., Haefele, S.M., Gamuyao, R., Ismail, A., Wissuwa, M and Heuer, S. 2010. Development and application of gene-based markers for the major rice QTL Phosphorus uptake 1. *Theoretical and Applied Genetics*. 120: 1073–1086.

- Chithrameenal, K., Alagarasan, G., Raveendran, M., Robin, S., Meena, S., Ramanathan, A and Ramalingam, J. 2018. Genetic enhancement of phosphorus starvation tolerance through marker assisted introgression of *OsPSTOL1* gene in rice genotypes harbouring bacterial blight and blast resistance. *PLoS ONE*. 13(9): e0204144. [https://doi.org/ 10.1371/journal.pone.0204144](https://doi.org/10.1371/journal.pone.0204144).
- Cho, Y., Victor, N, N., Xinbo, C., Kanokporn, T., Abdelmajid, K., Khalid, M., David, A., Lightfoot and Andrew, J. W. 2002. Quantitative Trait Loci Associated with Foliar Trigonelline Accumulation in *Glycine Max*. *Journal of Biomedicine Biotechnology*. 2(3): 151–157.
- Choudhary, A K., Pooniya, V., Bana, R.S., Kumar A and Singh, U. 2014. Mitigating pulse productivity constraints through phosphorus fertilization-a review. *Agricultural Reviews*. 35(4): 314 – 319.
- Clarke, J.M., De-Pauw, R.M and Townley-Smith, T.M. 1992. Evaluation of methods for quantification of drought tolerance in wheat. *Crop Science*. 32: 728-732.
- Clarkson, D.T. 1981. Nutrient interception and transport by root systems. In: Johnson CB (ed.) *Physiological processes limiting plant productivity*. London, Butterworths, pp 307-314.
- Collard, B.C.Y and Mackill, D.J. 2008. Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. *Philosophical Transactions of the Royal Society*. 363:557-572.
- Cordeiro, G.M., Christopher, M.J., Henry, R.J and Reinke, R.F. 2002. Identification of microsatellite markers for fragrance in rice by analysis of the rice genome sequence. *Molecular Breeding*. 9:245-250.
- Cordell, D., Drangert, J.O and White, S. 2009. The Story of Phosphorus: Global food security and food for thought. *Global Environmental Change*. 19:292–305.
- Correll, D.L. 1998. The role of phosphorus in the eutrophication of receiving waters: A Review. *Journal of Environment Quality*. 27:261–66.
- Courtois, B., Audebert, A., Dardou, A., Roques, S., Ghneim- Herrera, T., Droc, G., Frouin, J., Rouan, L., Gozé, E., Kilian, A., Ahmadi, N and Dingkuhn, M. 2013. Genome-Wide Association Mapping of Root Traits in a Japonica Rice Panel. *PLOS ONE* .8(11). e78037. doi:10.1371/journal.pone.0078037.

- Della Rovere, F., Fattorini, L., D'Angeli, S., Veloccia, A., Del Duca, S., Cai, G., Falasca, G and Altamura M. M. 2015. Arabidopsis SHR and SCR transcription factors and AUX1 auxin influx carrier control the switch between adventitious rooting and xylogenesis *in planta* and in *in vitro* cultured thin cell layers. *Annals of Botany*.115:617–628.
- Descalsota, G.I., Mallikarjuna Swamy, B.P., Zaw, H., Inabangan-Asilo, M., Amparado, A., Mauleon, R., Chadha-Mohanty, P., Arocena, E.C., Raghavan, C., Leung, H., Hernandez, J.E., Lalusin, A.B., Mendioro, M.S., Diaz, M.G and Reinke,R.2018. Genome-Wide Association Mapping in a Rice MAGIC Plus Population detects QTLs and Genes Useful for Bio- fortification. *Frontiers of Plant Science*. 9:1347.doi: 10.3389/fpls.2018.01347.
- Dey, P., Santhi, R., Maragatham, S and Sellamuthu, K.M. 2017.Status of Phosphorus and Potassium in the Indian Soils vis-à-vis World Soils. *Indian Journal of Fertilisers*. 13 (4): 44-59
- Dkhar, F., Rai, M and Tyagi, W. 2014. Looking Beyond *PSTOL1*: Marker development for two novel rice genes showing differential expression in P deficient conditions. *Journal of Genetics*. 93(2):573-577.
- Dobermann, A and Fairhurst, T. 2000. Rice: Nutrient disorders & nutrient management. Handbook series. *Potash & Phosphate Institute (PPI)*, Potash & Phosphate Institute of Canada (PPIC) and International Rice Research Institute. 191.
- Du, J., Zeng, Y., Pu, X.Y., Yang, S.M., Zhu, G.B and Wang, Y.C. 2008. Evaluation method and primary screening of high-phosphorus efficiency germplasm of Yunnan rice landraces. *Southwest China Journal of Agricultural Sciences*. 2: 10-35.
- Dwivedi, S.L., Crouch, J.H., Mackill, D.J., Xu, Y., Blair, M.W., Ragot, M., Upadhyaya, H.D and Ortiz, R. 2007. The molecularization of public sector crop breeding: Progress, problems, and prospects. *Advances in Agronomy*. 95: 163–318.
- Enoki, H., Sato, H and Kolhuma, K. 2002. SSR analysis of genetic diversity among maize inbred lines adapted for cold regions of Japan. *Theoretical and Applied Genetics*.104:1270 1277.
- European Commission. 2013. Consultative communication on the sustainable use of phosphorous.<http://ec.europa.eu/environment/consultations/pdf/phosphorus/EN.pdf>.

- Fageria N.K., Wright, R.J. and Baligar, V.C. 1988a. Rice cultivar evaluation for phosphorus use efficiency. *Journal of Plant Nutrition and Soil Science*. 111:105-109.
- Fageria, N.K and Baligar V.C.1997. Upland rice genotypes evaluation for phosphorus use efficiency. *Journal of Plant Nutrition*. 20:499–509.
- Fageria, N. K and Santos A.B.1998.Rice and common bean growth and nutrient concentration as influenced by aluminium on an acid lowland soil. *Journal of Plant Nutrition*. 21:903–912.
- Fageria, N.K and Filho, M.P.B. 2007. Dry-matter and grain yield, nutrient uptake, and phosphorus use-efficiency of lowland rice as influenced by phosphorus fertilization. *Communications in Soil Science and Plant Analysis*.38:1289-1297.
- Fageria, N.K., Morais, O.P., Baligar, V.C and Wright R.J.1988b. Response of rice cultivars to phosphorus supply on an oxisol. *Fertilizer Research*. 16: 195–206.
- Fageria, N.K and Santos, A.B. 2002. Lowland rice genotypes evaluation for phosphorus use efficiency. *Journal of Plant Nutrition*. 25(12):2793-2802.
- Falconer, D.S. 1989.Introduction to Quantitative Genetics.3rd edition. *John Wiley and Sons*: New York.
- Federici, M.T., Vaughan, D., Tomooka, N.,Kaga, A., Wang X., Doi, K., Francis, M., Zorrilla, G and Saldain, N. 2001. Analysis of Uruguayan weedy rice genetic diversity using AFLP molecular markers.*EJB Electronic Journal of Biotechnology*. 4(3).
- Feltus, F.A., Wan, J, Schulze, S.R, Estill, J.C, Jiang, N and Paterson, A.H. 2004. An SNP resource for rice genetics and breeding based on subspecies indica and japonica genome alignments. *Genome Res*. 14:1812–1819.
- Feng, M., Xianwu, Z., Guohu, M.I, Ping, H.E., Lihuang, Z and Fusuo, Z. 2000. Identification of quantitative trait loci affecting tolerance to low phosphorus in rice (*Oryza Sativa* L.). *Chinese Science Bulletin*. 45(6):520-525.
- Fernandez, G.C.J. 1992. Effective selection criteria for assessing plant stress tolerance. In:Proceeding of the *International Symposium, On adaptation of vegetables and other food crops in temperature and water stress*. Tqiwan: 257-270.
- Fernandez, M.E., Figueiras, A.M and Benito, C. 2002.The use of ISSR and RAPD marker for detecting DNA polymorphism, genotype identification and genetic diversity among barley cultivars with known origin. *Theoretical and Applied Genetics*. 104:845-851.

- Fisher, R. A and Maurer, R.1978. Drought resistance in spring wheat cultivars. I. Grain yield responses. *Australian Journal of Agricultural Research*. 29: 897-912.
- Fisher, R. A and Yate, F. 1963. Statistical tables for biological, agricultural and medical research. Oliv. Boyd, Endinburgh. *Fusarium udum* in pigeonpea. *Nematropica*, 19:12-28.
- Food and Agriculture Organization (FAO). 2015. The state of Food and Agriculture Social protection and agriculture: breaking the cycle of rural poverty. Rome: Food and Agriculture Organization of the United Nations.
- Food and Agriculture Organization (FAO). 2016. Food balance sheets average. Rome., Italy.
- Franza, S., Ehlerta, B., Liesea, A., Kurtha, J., Cazale, A and Romeisa, T.2011. Calcium-Dependent Protein Kinase CPK21 Functions in Abiotic Stress Response in *Arabidopsis thaliana*. *Molecular Plant*. 4(1):83–96.
- Fukrei, K.P., Kumar, A., Tyagi, W., Rai, M and Pattanayak, A. 2011. Genetic variability in yield and its components in upland rice grown in acid soils of North East India. *Journal of Rice Research*. 4(1 & 2): 4-7.
- Gamuyao, R., Chin, H.J., Tanaka, J.P., Pesaresi, P., Catausan, S., Dalid, Chery., Loedin, S.I., Mendoza, T.M.E., Wissuwa, M and Heuer, S. 2012. The protein kinase *Pstol1* from traditional rice confers tolerance of phosphorus deficiency. *Nature*.88:535-541.
- Garbers, C., DeLong, A., Deruere, J., Bernasconi, P and Soll, D.1996. A mutation in protein phosphatase 2A regulatory subunit A affects auxin transport in *Arabidopsis*. *The EMBO Journal*. 15(9):2115-2124.
- Garcia-Oliveira, A, L., Tan, L., Fu, Y and Sun, C. 2008. Genetic identification of Quantitative Trait Loci for Contents of mineral nutrients in rice grain. *Journal of Integrative Plant Biology*.1-9.
- Gavuzzi, P., Rizza, F., Palumbo, M., Campalino, R.G., Ricciardi, G.L. and Borghi, B. 1997. Evaluation of field and laboratory predictors of drought and heat tolerance in winter cereals. *Canadian Journal of Plant Science*. 77:523-531.
- Giri, J., Bhosale, R., Huang, G., Pandey, B. K., *et al.*, 2018. Rice auxin influx carrier OsAUX1 facilitates root hair elongation in response to low external phosphate. *Nature communications*.9: 1408.
- Golestani-Araghi, S and Assad, M.T. 1998. Evaluation of four screening techniques for drought resistance and their relationship to yield reduction ratio in wheat. *Euphytica*.103: 293-299.

- Guerriero, G., Hausman, J.F and Ezcurra, I.2015. WD40-Repeat Proteins in Plant Cell Wall Formation: Current Evidence and Research Prospects. *Frontiers of Plant Science*. 6:1112. doi: 10.3389/fpls.2015.01112.
- Gunther, F. 2005. A solution to the heap problem: the doubly balanced agriculture: integration with population. Available: <http://www.holon.se/folke/kurs/Distans/Ekofys/Recirk/Eng/balanced.shtml>.
- Gupta, A., Kakade, D.P., Singh, J., Janjal, P.H., Verulkar, S.B and Banerjee, S. 2016. Characterization of rice root transcriptome under Phosphorus Deficiency stress. *Int.J.Curr.Microbiol.App.Sci*. 5(8):273-279.
- Gu, K., Yang, B., Tian, D., Wu, L., Wang, D., Sreekala, C., Yang, F., Chu, Z., Wang, G.L., White, F.F and Yin, Z. 2005. R gene expression induced by a type-III effector triggers disease resistance in rice. *Nature*. 435:1122–1135.
- Haider, Z., Khan A.S and Zia S.2012.Correlation and Path Coefficient Analysis of Yield Components in Rice (*Oryza sativa* L.)Under Simulated Drought Stress Condition. *American-Eurasian J. Agric. & Environ. Sci*. 12 (1):100-104.
- Haldane, J.B.S. 1919. The combination of linkage values, and the calculation of distances between the loci of linked factors. *Journal of Genetics*. 8:299-309.
- Hasan, M.M., Rafii, M.Y., Ismail, M.R., Mahmood, M., Rahim, H.A, Alam, M.A and Latif, M.A.2015. Marker-assisted backcrossing: a useful method for rice improvement. *Biotechnology&BiotechnologicalEquipment*.29(2):237–254.
- Hasan, R.1996. Phosphorus status of soils in India. *Better Crops International*.10(2): 4-5.
- Heubl, G. 2010. New aspects of DNA-based authentication of Chinese medicinal plants by molecular biological techniques. *Planta Medica*. 76 (17):1963-1974.
- Heuer, S., Gaxiola, R., Schilling, R., Herrera-Estrella, L., Lopez-Arredondo, D., Wissuwa, M., Delhaize, E and Rouached, H. 2017.Improving phosphorus use efficiency: a complex trait with emerging opportunities. *The Plant Journal*. 90: 868–885.
- Heuer, S., Lu, X., Chin, J.H., Tanaka, J.P., Kanamori, H., Matsumoto, T., De Leon.T., Ulat, V.J., Ismail, A.M., Yano, M. 2009. Comparative sequence analyses of the major quantitative trait locus phosphorus uptake 1 (*Pup1*) reveal a complex genetic structure. *Plant Biotechnology Journal*. 7: 456–457.
- Hinsinger, P. 2001. Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. *Plant Soil*.237:173–81.

- Holford, I.C.R. 1997. Soil phosphorus: its measurements and its uptake by plants. *Australian Journal of Soil Research*. 35:227-239.
- Horii, H., Nemoto, K., Miyamoto, N and Harada, J. 2006. Quantitative trait loci for adventitious and lateral roots in rice. *Plant Breeding*. 125:198-200.
- Hu, B., Wu, P., Liao, C.Y., Zhang, W.P and Ni, J.J. 2001. QTLs and epistasis underlying activity of acid phosphatase under phosphorus sufficient and deficient condition in rice (*Oryza sativa* L.). *Plant and Soil*. 230:99-105.
- Huang, Y.F., Poland, J.A., Wight, C.P., Jackson, E.W and Tinker, N.A. 2014. Using genotyping-by-sequencing (GBS) for genomic discovery in cultivated oat. *PLoS ONE* :9, e102448; doi: 10.1371/journal.pone.0102448.
- Huang, N., Angeles, E.R., Domingo, J., Magpantay, G., Singh, S., Zhang, G., Kumaravadivel, N., Bennett, J and Khush, G.S. 1997. Pyramiding of bacterial blight resistance genes in rice: Marker-assisted selection using RFLP and PCR. *Theoretical Applied Genetics*.95: 313-320.
- Ishimaru K. 2003. Identification of a locus increasing rice yield and physiological analysis of its function. *Plant Physiology*.133: 1083-1090.
- Islam, M. A., Islam, M. R and Sarker, A. B. 2008.Effect of Phosphorus on Nutrient Uptake of *Japonica* and *Indica* Rice. *Journal of Agriculture and Rural Development*. 6(1&2):7-12.
- Ismail, A.M., Heuer, S., Thomson, J.T and Wissuwa, M. 2007. Genetic and genomic approaches to develop rice germplasm for problem soils. *Plant Molecular Biology*. 65:547-570.
- Jakkeral, S.A and Kajjidon, S.T. 2011. Understanding association pattern of root traits and seed yield components in enhancing phosphorus uptake under P- deficiency condition in blackgram (*Vignamungo* L.Hepper).*Karnataka Journal of Agricultural Sciences*. 24(4):440-443.
- Jansen, R.C. 1993. Interval mapping of multiple quantitative trait loci.*Genetics*.135:205-211.
- Jasinski, S.M. 2006. Phosphate Rock, Statistics and Information.US Geological Survey. <https://www.usgs.gov/centers/nmic/phosphate-rock-statistics-and-information>
- Jaynes, D.B., Kaspar, T.C., Colvin, T.S and James, E.D.2003. Cluster Analysis of Spatiotemporal Corn Yield Patterns in an Iowa Field. *Agronomy Journal*. 95:1-13.
- Jena, K.K and Mackill, D.J. 2008.Molecular Markers and their use in Marker-Assisted Selection in Rice. *Crop Science*. 48: 1266-1276.

- Jensen, T.L. 2010. Soil pH and the Availability of Plant Nutrients. *IPNI Plant Nutrition TODAY*. Fall, No. 2. 180. <http://www.ipni.net/publication/pntna.nsf/0/013f96e7280a696985257cd6006fb98f>
- Jewel, Z. A., Ali, J., Mahender, A., Hernandez, J., Pang Y and Li Z. 2019. Identification of Quantitative Trait Loci Associated with Nutrient Use Efficiency Traits, Using SNP Markers in an Early Backcross Population of Rice (*Oryza sativa* L.). *International Journal of Molecular Science*. 20: 1-27.
- Johnson, H.N., Robinson, H.F and Constock, R.E. 1955. Genotypic and phenotypic correlation in soybean and their implication in selection. *Agronomy Journal*. 47(10):477-483.
- Kamoshita, A., Zhang, J., Siopongco, J., Sarkarung, S., Nguyen, H.T and Wade, L.J. 2002. Effects of phenotyping environment on identification of QTL for rice root morphology under anaerobic conditions. *Crop Science*. 42:255-265.
- Kanbar, A., Shashidhar, H.E and Hittalmani, S. 2002. Mapping of QTL associated with root and related traits in DH population of rice (*Oryza sativa* L.). *Indian Journal of Genetics*. 62(4):287-290.
- Kao, C.H., Zeng, Z.B and Teasdale, R.D. 1999. Multiple Interval Mapping for Quantitative Trait Loci. *Genetics*. 152(3):1203-16
- Karthikeyan, A., Varadarajan, D., Jain, A., Held, M., Carpita, N and Raghothama, K. 2007. Phosphate starvation responses are mediated by sugar signalling in *Arabidopsis*. *Planta*. 225:907–18.
- Kavanova, M., Lattanzi, F.A., Grimoldi, A.A and Schnyder H. 2006. Phosphorus Deficiency Decreases Cell Division and Elongation in Grass Leaves. *Plant Physiology*. 141:766–775.
- Khan, U and Mohammad F. 2016. Application of stress selection indices for assessment of nitrogen tolerance in wheat (*Triticum aestivum* L.). *The Journal of Animal & Plant Sciences*. 26(1): 201-210.
- Khush, G.S and Jena, K.K. 2009. Current status and future prospects for research on blast resistance in rice (*Oryza sativa* L.). In: Advances in Genetics, Genomics and Control of Rice Blast Disease. 1-10.
- Khush, G.S. 2005. What it will take to feed 5.0 billion rice consumers in 2030. *Plant Molecular Biology*. 59: 1-6.
- Kirk, G.J.D. 2005. The biogeochemistry of submerged soils. *European journal of soil science*. 56:273-280.

- Kochian, L.V., Hoekenga, O. and Pineros, M.A. 2004. How do crop plants tolerate acid soils? Mechanisms of aluminium tolerance and phosphorus efficiency. *Annual Review of Plant Biology*. 55:459-493.
- Koide, Y., Tanaka, J.P., Rose, T., Fukuo, A., Konisho, K., Yanagihara, S., Fukuta Y and Wiaauwa, M.2013. QTLs for phosphorus deficiency tolerance detected in upland NERICA varieties. *Plant Breeding*. 132:259–265.
- Kong, D., Li, M., Dong, Z., Ji, H and Li, X. 2015. Identification of TaWD40D, a wheat WD40 repeat-containing protein that is associated with plant tolerance to abiotic stresses. *Plant Cell Rep*. 34(3):395-410.
- Kosambi, D.D. 1944. The estimation of map distances from recombination values. *Annals of Eugenics*.12: 172-175.
- Krishnamurthy, P., Sreedevi, B., Ram, T and Latha, P.C. 2014. Evaluation of rice genotypes for phosphorus use efficiency under soil mineral stress conditions. *Journal of Rice Research*. 7 (1&2):53-61.
- Kumar, S., Banks, T.W and Cloutier, S. 2012. SNP Discovery through next-generation sequencing and its applications. *International Journal of Plant Genomics*.1-15.
- Kuczynska, A., Maria, S and Tadeusz, A. 2007. Methods to predict transgressive segregation in barley and other self-pollinated crops. *Journal of Applied Genetics*. 48(4): 321-328.
- Lander, E. S and Botstein, D. 1989. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics*. 121:185-199.
- Lavakush, Yadav J., Verma J P., Jaiswal D K and Kumar A. 2014. Evaluation of PGPR and different concentration of phosphorus level on plant growth, yield and nutrient content of rice (*Oryza sativa*). *Ecological Engineering*. 62:123–128.
- Li, Z., Xie, Y., Dai, A and Liu, L. 2009. Root and shoot traits responses to phosphorus deficiency and QTL analysis at seedling stage using introgression lines of rice. *Journal of Genetics and Genomics*. 36:173-183.
- Lim, J.H., Chung, I.M., Ryu, S.S., Park, M.R and Yun, S.J. 2003. Differential responses of rice acid phosphatase activities and isoforms to phosphorus deprivation. *Journal of biochemistry and molecular biology*. 36(6):597-602.
- Lin, H.X., Qian, H.R., Zhuang, J.Y., Lu, J., Min, S.K., Xiong, Z.M., Huang, N and Zheng, K.L.1996. RFLP mapping of QTLs for yield and related characters in rice (*Oryza sativa* L.).*Theoretical and Applied Genetics*. 92:920-927.

- Litt, M and Luty, J.A. 1989. A hyper variable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. *American Journal of Human Genetics*. 44:397-401.
- Lo'pez-Arredondo, D.L., Leyva-Gonz'alez, M.A., Gonz'alez-Morales, S.I., Lo'pez-Bucio, J and Herrera-Estrella, L. 2014. Phosphate Nutrition: Improving Low-Phosphate Tolerance in Crops. *Annual Review of Plant Biology*. 65:95-123.
- Lu, K.T., Lee, H.C., Liu, F.S., Lo, C.F and Lin, J.H. 2010. Identification of Ginseng radix in Chinese medicine preparations by nested PCR-DNA sequencing method and nested PCR-restriction fragment length polymorphism. *Journal of Food Drug Analysis*. 18 (1):58–63.
- Luo, X., Liu, J., Dai, L., Zhang, F., Wan, Y and Xie, J. 2017. Linkage map construction and QTL identification of P-deficiency tolerance in *Oryza rufipogon* Griff. at early seedling stage. *Euphytica*. 213:96.
- Lynch, J., Epstein, E., Lauchli, A and Weigt, G.E. 1990. An automated greenhouses and culture system suitable for studies of phosphorus nutrition. *Plant, Cell and Environment*. 13:547–554.
- MacDonald G.K, Bennetta, E.M, Potterc, P.A and Ramankutty N. 2011. Agronomic phosphorus imbalances across the world's croplands. *PNAS*. 108(7):3086–3091.
- Mamathashree C.M., Girijesh, G.K and Vinutha B.S. 2018. Phosphorus dynamics in different soils. *Journal of Pharmacognosy and Phytochemistry*. 7(1): 981-985.
- Mao, X., Zhang, H., Tian, S., Chang, X and Jing, R. 2010. TaSnRK2.4, an SNF1-type serine/threonine protein kinase of wheat (*Triticum aestivum* L.) confers enhanced multi stress tolerance in Arabidopsis. *Journal of Experimental Botany*. 61(3):683–696.
- Matsumoto, T., Wu, J.Z., Kanamori, H., Katayose, Y., Fujisawa, M., Namiki, N., Mizuno, H., Yamamoto, K., Antonio, B.A. and Baba, T. 2005. The map-based sequence of the rice genome. *Nature*. 436:793–800.
- McCouch, S.R., Cho, Y. G., Yano, M., Paul, E and Blinstrub, M. 1997. Report on QTL nomenclature. *Rice Genetics News letter*. 14: 11-13.
- McCouch, S.R., Teytelman, L., Xu, Y.B., Lobos, K.B., Clare, K., Walton, M., Fu, B.Y., Maghirang, R., Li, Z.K., Xing, Y.Z., Zhang, Q.F., Kono, I., Yano, M., Fjellstrom, R., DeClerck, G., Schneider, D., Cartinhour, S., Ware, D and Stein, L. 2002. Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). *DNA Research*. 6:199-207.

- Mehra, P., Pandey, B.K and Giri J. 2014.Genome-wide DNA polymorphisms in low Phosphate tolerant and sensitive rice genotypes. *Scientific Reports*.5:13090;DOI: 10.1038/srep13090.
- Mekonnen, M.M and Hoekstra A.Y. 2017. Global Anthropogenic Phosphorus Loads to Freshwater and Associated Grey Water Footprints and Water Pollution Levels: A High-Resolution Global Study. *Water Resources Research*.53. <https://doi.org/10.1002/2017WR020448>
- Meng, L., Li, H., Zhang, L and Wang, J. 2015. QTL IciMapping: Integrated software for genetic linkage map construction and quantitative trait locus mapping in biparental populations. *The Crop Journal*. 3(3):269-283.
- Mogga, M., Sibiya, J., Shimelis, H., Lamo, J and Yao, N.2018. Diversity analysis and genome-wide association studies of grain shape and eating quality traits in rice (*Oryza sativa* L.) using DArT markers. *PLoS ONE*.13(6):e0198012. <https://doi.org/10.1371/journal.pone.0198012>.
- Mohan, M., Nair, S., Bhagwat, A., Krishna, T.G., Yano, M., Bhatia, C. R and Sasaki, T. 1997.Genome mapping, molecular markers and marker-assisted selection in crop plants. *Molecular Breeding*.3:87-103.
- Mollasadeghi, V., Valizadeh , M., Shahryariand, R.A and Imani, A. 2011. Evaluation of end drought tolerance of 12 wheat genotypes by stress in dices. *Middle-East Journal of Scientific Research*. 7(2):241-247.
- Muhr, G.R., Dutta, N.P and Subramanoey S. 1965.Soil Testing in India.USAID, New Delhi, India.
- Mukherjee, A., Sarkar, S., Chakraborty, A. S., Yelne, R., Kavishetty, V., Biswas, T and Bhattacharyya, S. 2014.Phosphate acquisition efficiency and phosphate starvation tolerance locus (*PSTOL1*) in rice. *Journal of genetics*. 93(3):683-688.
- Murray, M.G and Thompson, W.F. 1980. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Research*.8:4321.
- Naqvi, N.I and Chattoo B.B.1996. Development of a sequence characterized amplified region (SCAR) based indirect selection method for a dominant blast-resistance gene in rice.*Genome*. 39(1):26-30.
- Nautiyal, C.S., Bhadauria S., Kumar P., Lal H., Mondal, Rand Verma, D. 2000. Stress induced phosphate solubilization in bacteria isolated from alkaline soils. *FEMS Microbiology Letters*. 182:291–296.

- Neelam, K., Thakur S., Neha.,Yadav I.S., Kumar, K., Salwinder S. Dhaliwal, S.S and Singh, K. 2017. Novel Alleles of Phosphorus-Starvation Tolerance 1 Gene (*PSTOL1*) from *Oryza rufipogon* Confers High Phosphorus Uptake Efficiency. *Frontiers in Plant Science*. 8:509. doi: 10.3389/fpls.2017.00509.
- Neeraja, C.N., Maghirang-Rodriguez, R., Pamplona, A., Heuer, S., Collard, B., Septiningsih, E., Vergara, G., Sanchez, D., Xu, K., Ismail, A and Mackill, D. 2007. A marker assisted backcross approach for developing submergence-tolerant rice cultivars. *Theoretical and Applied Genetics*.115:767-776.
- Nguyen, T.T., Klueva, N., Chamareck, V., Aarti, A., Magpantay, G., Millena, A.C.M., Pathan, M.S and Nguyen. H.T. 2004.Saturation mapping of QTL regions and identification of putative candidate genes for drought tolerance in rice. *Molecular Genetics Genomics*.272:35-46.
- Nguyen, T.L and Bui C.B. 2006.Mapping QTLS for Phosphorus deficiency tolerance in rice (*Oryza sativa* L.).*Omon Rice*. 14:9.
- Ni, J.J., Wu, P., Lou, A.C, and Tao, Q.N. 1998. Rice seedling tolerance to phosphorus stress of *Japonica* and *Indica* Rice. *Journal of Agriculture and Rural Development*. 6(1&2):7-12.
- Nord, E and Lynch, J. 2008. Delayed Reproduction in *Arabidopsis thaliana* Improves Fitness in Soil with Suboptimal P Availability. *Plant, Cell and Environment*. 31:1432-1441.
- Onyia, V.N., Okechukwu, E.C., Atugwu, A.I and Akpan, N.M. 2017. Genetic variability studies on twelve genotypes of rice (*Oryza sativa* L.) for growth and yield performance in South Eastern Nigeria. *Notulae Scientia Biologicae*. 9(1): 110-115.
- Oono, Y., Kawahara, Y., Yazawa, T., Kanamori, H., Kuramata, M., Yamagata, H., Hosokawa, S., Minami, H., Ishikwa, S., Wu, J., Antonio, B., Handa, H., Itoh, T and Matsumoto, T. 2013. Diversity in the complexity of phosphate starvation transcriptomes among rice cultivars based on RNA-Seq profiles. *Plant Molecular Biology*. 83:523-537.
- Panigrahy, M., NageswaraRao, D., Yugandhar, P., SravanRaju, N., Krishnamurthy, P., Voleti, S.R and Sarla, N. 2014. Hydroponic experiment for identification of tolerance traits developed by rice Nagina 22 mutants to low-phosphorus in field condition. *Archives of Agronomy and Soil Science*: 60(4): 565-576.

- Paran and Michelmore. 1993. Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. *Theoretical and Applied Genetics*. 85:985-993.
- Paterson, A.H., Tanksley, S.D and Sorrels, M.E. 1991. DNA markers in plant improvement. *Advances in Agronomy*. 46:39-90.
- Pariasca-Tanaka, J., Chin, J.H., Drame, K.N., Dalid, C., Heuer, S and Wissuwa, M . 2014. A novel allele of the P starvation tolerance gene OsPSTOL1 from African rice (*Oryza glaberrima* Steud) and its distribution in the genus *Oryza*. *Theoretical and Applied Genetics*. 127:1387-1398.
- Peret, B., Clement, M., Nussaume, L and Desnos, T. 2011. Root developmental adaptation to phosphate starvation: better safe than sorry. *Trends in Plant Science*. 16:442-450.
- Perrier, X and Jacquemoud-Collet, J.P. 2006. Darwin software. <http://darwin.cirad.fr/darwin>
- Phung, N.T., Mai, C.D., Mournet, P., Frouin, J., Droc, D., Ta, N.K., Jouannic, S., Thi, L., Do, V.N., Gantet, P and Courtois, B. 2014. Characterization of a panel of Vietnamese rice varieties using DArT and SNP markers for association mapping purposes. *BMC Plant Biology*. 14:371.
- Piper, C.S .1966. Soil and Plant Analysis. *Hans Publisher*, Bombay.
- Ping, M.U., Chao, H., Xia, L.J., Feng L.L and Chao L.Z. 2008. Yield trait variation and QTL mapping in a DH population of rice under phosphorus deficiency. *Acta Agronomica Sinica*. 34(7):1137-1142.
- Pooni, H. S., Coombs, D. J and Jinks, P. S. 1987. Detection of epistasis and linkage of interacting genes in the presence of reciprocal differences. *Heredity*. 58(2): 257-266.
- Powell, W., Gordon, C.M. and Provan, J. 1996. Polymorphism revealed by simple sequence repeats. *Trends in Plant Science*. 1: 215–222.
- Pradeepa, N., Priya, P.S., Prince, K.S.J., Kavitha, S., Poornima, R., Prabhakar, M.S and Babu, R.C. 2012. *In Silico* analysis of a consensus QTL for drought resistance in rice. *Online Journal of Bioinformatics*. 13: 1–13.
- Price, A. H., Cairns, J. E., Horton, P., Jones, H.G and Griffiths, H. 2002. Linking drought resistance in upland rice using a QTL approach: progress and new opportunities to integrate stomatal and mesophyll responses. *Journal of experimental Botany*. 53:989-1004.

- Price, A.H. 2006. Believe it or not, QTLs are accurate!. *TRENDS in Plant Science*. 11(5): 213-216.
- Provan, J.G., Corbott, J.W., Niol, Mc and Powell, W. 1997. Chloroplast DNA variability in wild and cultivated rice (*Oryza sativa*. L) revealed by polymorphic chloroplast simple sequence repeats. *Genome*. 40:104-110.
- Qu, Y., Mu, P., Zhang, H., Chen, C. Y., Gao, Y., Tian, Y., Wen, F and Li, Z. 2008. Mapping QTL of root morphological traits at different growth stages in rice. *Genetica*. 133: 187-200.
- Raghothama, K.G. 1999. Phosphate acquisition. *Annual Review of Plant Physiology and Plant Molecular Biology*. 50:665-693.
- Rahman, S.N., Islam, M.S., Alam, M.S and Nasiruddin, K.M. 2006. Genetic polymorphism in rice (*Oryza sativa* L.) through RAPD analysis. *Indian Journal of Biotechnology*. 6: 224-229.
- Rameeh, V. 2015. Nitrogen deficiency stress indices of seed yield in rapeseed (*Brassica napus* L.) genotypes. *Cercetari Agronomice in Moldova*. 158 (1/161): 89-96.
- Rao, I.M., Borrero, V., Ricaurte, J., Garcia, R and Ayarza, M.A. 1996. Adaptive attributes of tropical forage species to acid soils. II. Differences in shoot and root growth responses to varying phosphorus supply and soil type. *Journal of Plant Nutrition*. 19:323-352.
- Rejesus, R.M., Mohanty, S and Balagtas, J.V. 2012. Forecasting global rice consumption, Department of Agricultural and Resource Economics, North Carolina State University.
- Rodriguez, D., Pomar M.C and Goudriaan J. 1998. Leaf primordia initiation, leaf emergence and tillering in wheat (*Triticum aestivum* L.) grown under low-phosphorus conditions. *Plant and Soil*. 202(1):149-157.
- Rouached, H., Arpat, A.B and Poirier, Y. 2013. Regulation of Phosphate Starvation responses in Plants: Signaling Players and Cross-Talks. *Molecular Plant*. 3(2):288-299.
- Rose, T.J and Wissuwa, M. 2012. Rethinking internal phosphorus utilization efficiency: a new approach is needed to improve PUE in grain crops. *Advances in Agronomy*. 116:183-215.
- Rosielle, A.A and Hamblin. J. 1981. Theoretical aspect of selection for yield in stress and non-stress environment. *Crop Science*. 21: 943-946.
- Rosmarin, A. 2004. The Precarious Geopolitics of Phosphorous, Down to Earth: *Science and Environment Fortnightly*. 27-31.

- Saengprajak, J. 2012. Development of a sequence characterized amplified region (SCAR) marker for identification of Thai jasmine rice (*Oryza sativa* L.) variety 'KDML105'. *African Journal of Biotechnology*. 11(59): 12262-12268.
- Sama, V.S.A.K., Himabindu, K., Bhaskar Naik, S., Sundaram, R.M., Viraktamath, B.C and Bentur, J.S. 2012. Mapping and marker-assisted breeding of a gene allelic to the major Asian rice gall midge resistance gene *Gm8*. *Euphytica*.doi: 10.1007/s10681-012-0724-y.
- Sanchez, P.A and Salinas, J.G. 1981. Low-input technology for managing oxisols and ultisols in tropical America. *Advances in Agronomy*. 34:279-406.
- Sarkar, S., Yelne, R., Chatterjee, M., Das, P., Debnath S., Chakraborty A., Mandal, N., Bhattacharya, K and Bhattacharyya, S. 2011. Screening for phosphorus(P) tolerance and validation of Pup-1 linked markers in indica rice. *Indian Journal of Genetics and Plant breeding*. 71(3):209-213.
- Schindler, D.W, Carpenter, S.R, Chapra, S.C, Hecky, R.E and Orihel, D.M. 2016. Reducing phosphorus to curb lake eutrophication is a success. *Environ Sci Technol*. 50:8923-8929.
- Sebastian, L.S., Hipolito, L.R and Gracia, J.S. 1998. Molecular markers analysis of the contribution made by landraces to modern Philippine rice cultivars. *SABRAO Journal of Breeding and Genetics*. 30: 73-81.
- Shen, J., Yuan, L., Zhang, J., Li, H., Bai, Z., Chen, X., Zhang, W and Zhang, F. 2011. Phosphorus Dynamics: From Soil to Plant. *Plant Physiology*. 156:997–1005.
- Shepherd, K.D., Cooper, P.J.M., Allan, A.Y., Drennan, D.S.H and Keatinge, J.D.H. 1987. Growth, water-use and yield of barley in Mediterranean-type environments. *Journal of Agricultural Science*. 108:365-378.
- Shimizu A., Kato, K, Komatsu, A., Motomura, K and Ikehashi, H. 2008. Genetic analysis of root elongation induced by phosphorus deficiency in rice (*Oryza Sativa* L.): fin QTL mapping and multivariate analysis of related traits. *Theoretical and Applied Genetics*. 117:987-996.
- Shimizu, A., Yanagihara, S., Kawasaki, S and Ikehashi, H. 2004. Phosphorus deficiency induced root elongation and its QTL in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics*. 109(7):1361-1368.
- Singh, S., Sengar, R.S., Kulshreshtha, N., Datta, D., Tomar, R.S., Rao, V.P., Garg, D and Ojha, A. 2015. Assessment of multiple tolerance indices for salinity stress in bread wheat (*Triticum aestivum* L.). *Journal of Agricultural Science*. 7(3):49-57.

- Sivasubramanian, S and Madhavamenon, P. 1973. Genotypic and phenotypic variability in rice. *The Madras Agricultural Journal*. 60:1093-1096.
- Smith, F.W. 2001. Plant response to nutritional stresses. In: Hawkesford MJ, Buchner P (eds.) *Molecular analysis of plant adaptation to the environment*. Kluwer Academic Publishers. The Netherlands. 249-269.
- Soltan, S., Römer, W., Adgo, E., Gerke, J and Schilling, G. 1993. Phosphorus absorption by Egyptian, Ethiopian and German soils and P uptake by rye (*Secale cereale* L.) seedlings. *Journal of Plant Nutrition and Soil Science* .156:501-506.
- Souframanien, J., Manjaya, J.G., Krishna, T.G and Pawar, S.E. 2003. Random amplified polymorphic DNA analyses of cytoplasmic male sterile and male fertile Pigeonpea (*Cajanus cajan* L.) Millsp.). *Euphytica*. 129:293-299.
- Spartz, A.K., Ren, H., Park, M.Y., Grandt, K.N., Lee, S.H., Murphy, A.S., Sussman, M.R., Overvoorde, P.J and Graya, W.M. 2014. SAUR Inhibition of PP2C-D Phosphatases Activates Plasma Membrane H⁺-ATPases to Promote Cell Expansion in Arabidopsis. *The Plant Cell*. 26:2129–2142.
- Sreelakshmi, C.H., Sameer, Kumar, C.V and Shivani, D. 2010. Genetic analysis of yield and its component traits in drought tolerant genotypes of Pigeonpea (*Cajanus cajan* L. Millspaugh). *Electronic Journal of Plant Breeding*. 1(6):1488-1491.
- Stam, P. 1993. Construction of integrated genetic linkage maps by means of a new computer package Join Map. *Plant Journal*. 3:739-744.
- Steen, I. 1998. Phosphorus availability in the 21st Century: management of a nonrenewable stress tolerance: a transcriptome analysis of two rice lines contrasting in tolerance to phosphorus deficiency. *Journal of Rice*. 2(4):167-185.
- Sundaram, R.M., Vishnupriya, M.R., Biradar, S.K., Laha, G.S., Reddy, A.G., Rani, N.S., Sharma, N. P and Sonti, R.V. 2008. Marker assisted introgression of bacterial blight resistance in Samba Mahsuri, an elite *indica* rice variety. *Euphytica*. 160: 411-422.
- Swamy Mahadeva, H.K., Anila, M., Kale, R.R., Bhadana, V.P., Anantha, M.S., Brajendra, P., Hajira, S.K., Balachiranjeevi, C.H., Laxmi Prasanna, B., Pranathi, K., Dilip, T., Bhaskar, S., Abhilash Kumar, V., Kousik, M.B.V.N., Harika, G., Swapnil, K., Rekha, G., Cheralu, C., Gouri Shankar, V., Narendra Reddy, S., Kumar, S., Balachnadran, S.M., Madhav, M.S., Mahendra Kumar, R and Sundaram, R.M. 2019. Phenotypic and molecular characterization of rice

germplasm lines and identification of novel source for low soil phosphorus tolerance in rice. *Euphytica*.215:18.

- Swings, J., Van den Mooter, M., Vauterin, L., Hoste, B., Gillis, M., Mew, T.W and Kersters, K. 1990. Reclassification of the causal agents of bacterial blight (*Xanthomonas campestris* sp. *oryzae*) and bacterial leaf streak (*Xanthomonas campestris* sp. *oryzicola*) of rice as pathovars of *Xanthomonas oryzae* (ex Ishiyama 1922) sp. nov., nom. rev. *International Journal of Systematic Bacteriology*. 40: 309-311.
- Syers, J.K., Johnston, A.E and Curtin, D. 2008. Efficiency of Soil and Fertilizer Phosphorus: Reconciling Changing Concepts of Soil Phosphorus Behavior with Agronomic Information. *FAO Fertilizer and Plant Nutrition Bulletin*. 18:108. (FAO: Rome).
- Tanaka, P.J., Satoh, K., Rose, T., Mauleon, R and Wissuwa, M. 2009. Stress response versus stress tolerance: a transcriptome analysis of two rice lines contrasting in tolerance to phosphorus deficiency. *Journal of Rice*. 2(4):167-185.
- Tadano, T., Ozawa, K., Sakai, H., Osaki, M and Matsui, H. 1993. Secretion of acid phosphatase by the roots of crop plants under phosphorus-deficient conditions and some properties of the enzyme secreted by lupin roots. *Plant Soil*. 156:95-98.
- Takehisa, H., Sato, Y., Antonio, B.A and Nagamura, Y. 2013. Global transcriptome profile of rice root in response to essential macronutrient deficiency. *Plant Signaling and Behavior*. 8: e24409; <http://dx.doi.org/10.4161/psb.24409>.
- Tanksley, S. D and Nelson, J. C. 1996. Advanced backcross QTL analysis: a method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. *Theoretical and Applied Genetics*.92:191-203.
- Tautz, D.M., Trick and Dover, G.A. 1986. Cryptic simplicity in DNA is a major source of genetic variation. *Nature*. 322:652-656.
- Teng, W., Deng, Y., Chen, X. P., Xu, X.F, Chen, R.Y., Lv, Y., Zhao, Y., Zhao, X.Q., He, X., Li, B., Tong, Y.P., Zhang, F.S and Li, Z.S. 2013. Characterization of root response to phosphorus supply from morphology to gene analysis in field-grown wheat. *Journal of Experimental Botany*. 64(5):1403-1411.
- Thakur, R.K. 2014. Determination of phosphorus in soil and plant. In Thakur, R.K , Baghel, S.S., Sharma G.D., Sahu, P.C and Amule, P.C (ed.). Laboratory manual. Centre for advanced faculty training. JNKVV, Jabalpur, MP. 20-22.

- Tian, J and Liao, H. 2015. The role of intracellular and secreted purple acid phosphatases in plant phosphorous scavenging and recycling. *In Annual Plant Reviews*. 48: 265-287.
- Tian, Z., Li, J., He, X., Jia, X., Yang, F. and Wang, Z. 2017. Grain yield, dry weight and phosphorus accumulation and translocation in two rice (*Oryza sativa* L.) varieties as affected by salt-alkali and phosphorus. *Sustainability*. 9:1461;doi:10.3390/su9081461.
- Tilman, D., Cassman, K.G., Matson, P.A., Naylor, R and Polasky, S. 2002. Agricultural sustainability and intensive production practices. *Nature*. 418:671-677.
- Tiwari, K.N. 2001. Phosphorus Needs of Indian Soils and Crops. *Better Crops International*. 15(2):23.
- Tiwari, V. K., Rawat, N., Chhuneja, P., Neelam, K., Aggarwal, R and Randhawa, G. S. 2009. Mapping of quantitative trait loci for grain iron and zinc concentration in diploid A genome wheat. *Journal of Heredity*. 100:771-776.
- Toojinda, T., Baird, E., Booth, A., Broers, L., Hayes, P., Powell, W., Thomas, W., Vivar, H and Young, G. 1998. Introgression of quantitative trait loci (QTLs) determining stripe rust resistance in barley: an example of marker-assisted line development. *Theoretical Applied Genetics*. 96:123-131.
- Tyagi, W and Rai, M. 2016. Root transcriptome of two acidic soil adapted Indica rice genotypes suggest diverse and complex mechanism of low phosphorus tolerance. *Protoplasma*. 1-12.
- Tyagi, W., Rai, M and Dohling, A.K. 2012. Haplotype analysis for Pup1 locus in rice genotypes of North-Eastern and Eastern India to identify suitable donors tolerant to low phosphorus. *SABRAO Journal of Breeding and Genetics*. 44:398-405.
- Van Nguyen., Nguu and Ferrero, A. 2006. Meeting the challenges of global rice production. *Paddy and Water Environment*. 4.1-9. 10.1007/s10333-005-0031-5.
- Vance, C. 2010. Quantitative trait loci, epigenetics, sugars and MicroRNAs: Quaternaries in phosphate acquisition and use. *Plant physiology*. 154:582-588.
- Vance, C.P. 2001. Symbiotic nitrogen fixation and P acquisition plant nutrition a world of declining renewal resources. *Plant Physiology*. 127:390-397.
- Vance, C.P., Uhde-Stone, C and Allan, D.L. 2003. Phosphorus acquisition and use: critical adaptations by plants for securing a non-renewable resource. *New Phytologist*. 157:423-47.

- Vandamme, E., Wissuwa, M., Rose, T., Dieng, I., Drame, K.N., Fofana, M., Senthilkumar, K., Venuprasad, R., Jallow, D., Segda, Z., Suriyagoda, L., Sirisena, D., Kato, Y and Saito, K. 2016. Genotypic variation in grain p loading across diverse rice growing environments and implications for field P balances. *Frontiers in Plant Science*.7:1435; doi:10.3389/fpls.2016.01435.
- Vejchasarn, P., Lynch, J.P and Brown, K.M. 2016. Genetic variability in phosphorus responses of rice root phenotypes. *Rice*.9:29. DOI 10.1186/s12284-016-0102-9.
- Vellaikumar, S. and Malarvizhi P. 2017.Application of pup1 markers for identification of rice (*Oryza Sativa* L.) cultivars with tolerance to phosphorus deficiency. *International Journal of Agricultural Science and Research*. 7(4):713-718.
- Verdu, C.F., Guyot, S., Childebrand, N., Bahut, M., Celton, J.M., Gaillard, S., Lasserre-Zuber, P., Troglio, M., Guilet, D. and Laurens, F. 2014. QTL analysis and candidate gene mapping for the polyphenol content in cider apple. *PLoS ONE*. 9(10): p.e107103.
- Vierling, R.A and Nguyen, H. 1992. Use of RAPD markers to determine the genetic diversity of diploid wheat genotypes.*Theoretical and Applied Genetics*.84:835-838.
- Vigueira, C.C., Small,L.L and Olsen, K.M. 2016. Long-term balancing selection at the Phosphorus Starvation Tolerance 1 (PSTOL1) locus in wild, domesticated and weedy rice (*Oryza*). *BMC Plant Biology*.16:101.
- Vlek, P.L.G and Koch, H. 1992. The soil resource base and food production in the developing world: special focus on Africa. *Gättinger Beitragezur Landund Forstwirtschaft in den Tropen und Subtropen*. 71:139-160.
- Vos, P., Hogers, R., Bleeker, M., Reijans, M., Van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M and Zabeau, M. 1995. AFLP: A new technique for DNA fingerprinting. *Nucleic Acids Research*. 23:4407-4414.
- Wang, C., Ying, S., Huang, H., Li, K., Wu, P and Shou, H. 2009a.Involvement of OsSPX1 in phosphate homeostasis in rice. *The Plant Journal*. 57:895–904.
- Wang, A., Chen. F., Zhang, F and Guohua M. 2010.Two strategies for achieving higher yield under phosphorus deficiency in winter wheat grown in field conditions. *Field Crops Research*.118: 36-42.
- Wang, J., Pei, L., Jin, Z., Zhang, K and Zhang, J. 2016. Over expression of the protein phosphatase 2A regulatory subunit a gene *ZmPP2AA1* improves low phosphate tolerance by remodeling the root system architecture of maize. *PLoS ONE* 12(4):e0176538. <https://doi.org/10.1371/journal>.

- Wang, K., Cui, K., Liu, G., Luo, X., Huang, J., Nie, L., Wei, D and Peng, S. 2017. Low straw phosphorus concentration is beneficial for high phosphorus use efficiency for grain production in rice recombinant inbred lines. *Field Crops Research*. 203:65-73.
- Wang, K., Cui, K., Liu, G., Xie, W., Yu, H., Pan, J., Huang, J., Nie, L., Shah, F and Peng, S. 2014. Identification of quantitative trait loci for phosphorus use efficiency traits in rice using a high density SNP map. *BMC Genetics*.15:155. DOI 10.1186/s12863-014-0155-y.
- Wang, S., Basten, C and Zeng, Z. 2005a. Windows QTL Cartographer Version 2.5, Raleigh, NC: Department of Statistics. North Carolina State University.
- Wang, X.S., Zhu, J., Mansueto, L. and Bruskiewich, R. 2005b. Identification of candidate genes for drought stress tolerance in rice by the integration of a genetic (QTL) map with the rice genome physical map. *Journal-Zhejiang University Science*. 6(5):382.
- Wang, Z., Han, H., Huang, H., Duan, K., Zhongchang, W and Wu, P. 2009b. Regulation of *OsSPX1* and *OsSPX3* on Expression of *OsSPX* domain Genes and Pi starvation SIGNALING in Rice. *Journal of Integrative Plant Biology*. 51(7): 663-674.
- Wani, S.H and Sah K.S.2014. Biotechnology and Abiotic Stress Tolerance in Rice. *Rice Research: Open Access*.2(2).doi: 10.41 72/jrr.1000e105.
- Webeck, E., Matsubae, K., Nakajima, K., Nansai, K and Nagasaka, T. 2014. Analysis of phosphorus dependency in Asia. *Socio technica*. 11: 119-126.
- Weber and Moorthi, B. R. 1952. Heritable and non-heritable relationship and variability of oil content and agronomic characteristics in the F₂ generation of soybean crosses. *Agronomy Journal*. 44 :202-209.
- Williams. J.G.K., Kubelik, A.R., Livak, K.J., Rafalsky, J.A and Tingey, S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research*. 18:6531-6535.
- Williamson, L.C., Ribrioux, S.P.C.P., Fitter, A.H and Leyser, H.M.O. 2001. Phosphate availability regulates root system architecture in Arabidopsis. *Plant Physiology*. 126:875-882.
- Winter, J.M and Moore, B.S. 2009. Exploring the Chemistry and Biology of Vanadium-dependent Haloperoxidases. *Journal of Biological Chemistry*. 284(28).18577-18581.

- Wissuwa, M and Ae, N. 2001. Genotypic variation for tolerance to phosphorus deficiency in rice and the potential for its exploitation in rice improvement. *Plant Breeding*. 120: 43-48.
- Wissuwa, M. 2003. How Do Plants Achieve Tolerance to Phosphorus Deficiency? Small Causes with Big Effects. *Journal of Plant Physiology*. 133:1947-1958.
- Wissuwa, M. 2005. Combining a modelling with a genetic approach in establishing associations between genetic and physiological effects in relation to phosphorus uptake. *Plant and Soil*. 269: 57-68.
- Wissuwa, M., Mazzola, M and Picard, C. 2009. Novel approaches in plant breeding for rhizosphere-related traits. *Plant and Soil*. 321:409-430.
- Wissuwa, M., Wegner, J., Ae, N and Yano, M. 2002. Substitution mapping of Pup1: a major QTL increasing phosphorus uptake of rice from a phosphorus-deficient soil. *Theoretical and Applied Genetics*. 105:890-897.
- Wissuwa, M., Yano, M and Ae, N. 1998. Mapping of QTLs for phosphorus deficiency tolerance in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics*. 97:777-783.
- Wu, P., Shou, H., Xu, G and Lian, X. 2013. Improvement of phosphorus efficiency in rice on the basis of understanding phosphate signaling and homeostasis. *Journal of Current Opinion in Plant Biology*. 16:205-212.
- Xu, Y. 2010. Molecular plant breeding. CAB International. Wallingford, Oxfordshire. [https://www.scirp.org/\(S\(vtj3fa45qm1ean45vvffcz55\)\)/reference/ReferencesPapers.aspx?ReferenceID=1463036](https://www.scirp.org/(S(vtj3fa45qm1ean45vvffcz55))/reference/ReferencesPapers.aspx?ReferenceID=1463036)
- Xu, K., Deb, R and Mackill, D.J. 2004. A Microsatellite Marker and a Co-dominant PCR Based Marker for Marker-Assisted Selection of Submergence Tolerance in Rice. *Crop Science*. 44:248-253.
- Yadav, S., Anuradha, G., Kumar, R.R., Vemireddy, L.R., Sudhakar, R., Donempudi, K., Venkata, D., Jabeen, F., Narasimhan, Y.K., Marathi, B and Siddiq, E.A. 2015. Identification of QTL and possible candidate genes conferring sheath blight resistance in rice (*Oryza sativa* L.). *SpringerPlus*. 4(1):1.
- Yadav, S.K., Suresh, B.G., Pandey, P. and Kumar. B. 2010. Assessment of genetic variability, correlation and path association in rice (*Oryza sativa* L.). *Journal of Bio-Science*. 18:1-8.
- Yang X, Post W. M, Thornton P E, and Jain A. 2013. The distribution of soil phosphorus for global biogeochemical modeling. *Biogeosciences*. 10: 2525-2537.

- Yasala, A.K., Rawat, N., Kumar Sama., V.A.S., Kudapa, H., Sundaram, R.M and Bentur J. S. 2012.*In silico* analysis for gene content in rice genomic regions mapped for the gall midge resistance genes. *Plants Omics Journal*. 5(4):405-413
- Yaseen, M and. Malhi, S.S. 2009.Differential growth performance of 15 wheat genotypes for grain yield and phosphorus uptake n a low phosphorus soil without and with applied phosphorus fertilizer. *Journal of Plant Nutrition*. 32: 1015-1043.
- Yoon, D. B., Kang, K. H., Kim, H. J., Ju, H. G., Kwon, S. J and Suh, J. P. 2006. Mapping quantitative trait loci for yield components and morphological traits in an advanced backcross population between *Oryza grandiglumis* and *O. sativa* japonica cultivar Hwaseongbyeon. *Theoretical and applied genetics*.12:1052-1062.
- Yu, H., Xie, W., Li, J., Zhou, F and Zhang, Q. 2014. A whole-genome SNP array (RICE6K) for genomic breeding in rice. *Plant Biotechnology Journal*. 12:28–37.
- Yumnam, J S., Rai, M and Tyagi W. 2015.Allele mining across two low-P tolerant genes PSTOL1 and PupK20-2 reveals novel haplotypes in rice genotypes adapted to acidic soils. *Plant Genetic Resources: Characterization and utilization*: 1-9.
- Zabeau, M and Vos, P. 1993. Selective restriction fragment amplification: a general method for DNA fingerprinting. Publication 0 534 858 A1, bulletin 93/13.European Patent Office, Munich, Germany.
- Zang, D., Li, H., Xu, H., Zhang, W., Zhang, Y., Shi, X and Wang, Y.2016. An Arabidopsis Zinc Finger Protein Increases Abiotic Stress Tolerance by Regulating Sodium and Potassium Homeostasis, Reactive Oxygen Species Scavenging and Osmotic Potential. *Frontiers in Plant Science*. 7:1272. doi: 10.3389/fpls.2016.01272.
- Zeng, Z. B. 1994. Precision Mapping of Quantitative Trait Loci. *Genetics*.136:1457-1468.
- Zhang, D., Song, H., Cheng, H., Hao, D., Wang, H and Kan, G. 2014. The acid phosphate seen coding gene GmACP1 contributes to soybean tolerance to low-phosphorus stress.*PLoS Genetics*. 10:e1004061.
- Zhang, Q., Lin, S.C., Zhao, B.Y., Wang, C.L., Yang, W.C., Zhou, Y.I., Li, D.Y., Chen, C.B and Zhu, L.H. 1998. Identification and Tagging a new gene for resistance to bacterial blight (*Xanthomonas oryzae*pv. *oryzae*) from *O. rufipogon*. *Rice Genetics NewsLetter*.15: 138-141.

- Zhang, Y., Anis, G B., Wang, R., Wu, W., Yu, N., Shen X., Zhan X., Cheng S and Cao L.2017. Genetic dissection of QTL against phosphate deficiency in the hybrid rice 'Xieuo9308'. *Plant Growth Regulator*.1-11.
- Zhao, K., Tung, C.W., Eizenga, G.C., Wright, M.H., Ali, M.L., Price, A.H., Norton, G.J., Islam, M.R., Reynolds, A., Mezey, J., et al. 2011. Genome-wide association mapping reveals a rich genetic architecture of complex traits in *Oryza sativa*. *Nature Communications*. 2:467.
- Zhou, T., Du, Y., Ahmed, S., Liu, T., Ren, M., Liu, M., and Yang, W. 2016. Genotypic differences in phosphorus efficiency and the performance of physiological characteristics in response to low phosphorus stress of soybean in southwest of china. *Frontiers in Plant Science*. 7: 1776; doi: 10.3389/fpls.2016.01776.
- Zhu, J., Gale,M.D., Quarrie, S., Jackson, M.T and Bryan, G.J.1998. AFLP markers for the study of rice biodiversity. *Theoretical and Applied Genetics*. 96(5):602-611.

Appendices

Appendix A

Overall Performance of 112 RILs along with their parents for various agro-morphological and yield related traits under low soil P condition

Genotypes	PH	SL	NPT	PL	RL	RV	DSW	DRW	SY	1000 SW	DFP	Biomass	r/s ratio	P/seed
wazuhophek	73.00	68.00	8.00	18.00	26.00	30.00	5.20	2.50	9.00	22.1	134.00	14.20	0.49	0.50
ISM	51.00	48.00	2.00	11.00	22.00	10.00	2.00	0.20	2.50	11.00	122.00	4.50	0.10	0.09
KR-2	85.00	79.00	4.00	19.00	25.00	15.00	8.10	1.30	2.50	19.50	127.00	10.60	0.16	0.53
KR-3	88.00	78.00	5.00	18.00	24.00	10.00	6.60	1.00	9.00	16.40	128.00	15.50	0.15	0.77
KR-4	91.00	87.00	3.00	17.00	21.00	13.00	9.00	2.60	8.20	19.70	129.00	17.10	0.29	0.85
KR-5	92.00	82.00	3.00	18.00	25.00	25.00	12.00	0.65	4.10	18.00	127.00	16.10	0.05	0.80
KR-6	70.00	68.00	4.00	15.00	27.00	20.00	9.70	1.15	2.20	18.50	130.00	11.80	0.12	0.59
KR-8	63.00	59.00	4.00	14.00	26.00	20.00	6.80	1.50	1.70	15.50	128.00	8.40	0.22	0.42
KR-9	67.00	56.00	3.00	11.00	20.00	20.00	4.50	0.75	1.20	18.10	129.00	5.70	0.17	0.28
KR-10	70.00	58.00	2.00	16.00	24.00	13.00	3.00	0.55	0.60	12.70	130.00	3.60	0.19	0.17
KR-11	83.00	65.00	5.00	16.00	29.00	15.00	11.90	2.00	5.00	21.70	131.00	16.90	0.17	0.84
KR-13	68.00	53.00	5.00	14.00	22.00	8.00	3.70	0.60	1.70	16.50	134.00	5.40	0.16	0.26
KR-14	66.00	61.00	3.00	14.00	25.00	8.00	4.50	0.80	1.90	16.40	128.00	6.40	0.18	0.32
KR-16	57.00	37.00	1.00	9.00	22.00	5.00	3.50	0.65	1.10	16.30	130.00	4.60	0.19	0.23
KR-18	70.00	61.00	4.00	11.00	21.00	10.00	5.30	0.75	1.50	15.20	128.00	6.80	0.14	0.34
KR-19	65.00	39.00	1.00	9.00	22.00	8.00	2.10	0.40	0.20	15.00	130.00	2.30	0.19	0.00
KR-20	61.00	52.00	3.00	12.00	26.00	10.00	4.10	0.60	2.20	15.70	129.00	6.30	0.15	0.31
KR-21	68.00	50.00	7.00	15.00	21.00	10.00	3.60	1.00	4.80	15.00	130.00	8.30	0.28	0.41
KR-23	65.00	60.00	5.00	17.00	17.00	10.00	2.40	0.50	2.10	15.50	124.00	4.50	0.21	0.22
KR-24	53.00	46.00	2.00	10.00	18.00	8.00	1.70	0.25	0.80	12.30	128.00	2.50	0.15	0.12
KR-25	50.00	40.00	2.00	8.00	24.00	5.00	1.90	0.45	0.70	13.60	126.00	2.60	0.24	0.13
KR-26	54.00	56.00	2.00	8.00	27.00	10.00	3.60	0.85	0.30	12.50	130.00	3.90	0.24	0.19
KR-27	60.00	41.00	2.00	7.00	20.00	13.00	2.80	0.65	0.70	14.30	126.00	3.50	0.23	0.17
KR-31	57.00	51.00	4.00	10.00	21.00	10.00	3.20	0.50	1.60	13.50	129.00	4.70	0.16	0.23
KR-32	54.00	49.00	4.00	11.00	17.00	10.00	2.50	0.40	1.50	13.70	128.00	4.00	0.16	0.20
KR-38	62.00	45.00	1.00	9.00	21.00	20.00	5.40	1.15	0.40	12.00	130.00	5.70	0.21	0.28
KR-39	70.00	61.00	5.00	13.00	14.00	10.00	3.90	0.35	1.40	17.00	129.00	5.30	0.09	0.26
KR-42	40.00	37.00	2.00	7.00	18.00	13.00	1.20	0.15	0.60	12.00	129.00	1.80	0.13	0.08
KR-45	66.00	50.00	2.00	10.00	17.00	10.00	6.20	0.80	0.30	10.00	129.00	6.40	0.13	0.32
KR-46	43.00	37.00	2.00	8.00	18.00	5.00	0.90	0.10	0.20	10.00	126.00	1.10	0.11	0.00
KR-49	69.00	64.00	2.00	11.00	26.00	18.00	5.30	0.90	0.60	15.60	129.00	5.90	0.17	0.29
KR-58	64.00	61.00	4.00	13.00	25.00	20.00	8.60	1.50	1.30	15.20	126.00	9.80	0.18	0.00
KR-65	59.00	43.00	2.00	10.00	23.00	15.00	2.90	0.30	0.20	14.20	121.00	3.10	0.10	0.15
KR-67	57.00	47.00	4.00	12.00	19.00	13.00	1.80	0.35	1.30	13.50	126.00	3.10	0.20	0.15

KR-70	73.00	67.00	6.00	16.00	24.00	25.00	7.50	1.70	7.20	17.70	126.00	14.70	0.23	0.73
KR-71	80.00	76.00	5.00	19.00	25.00	25.00	9.00	1.85	8.00	21.00	129.00	16.90	0.21	0.84
KR-73	78.00	73.00	5.00	17.00	21.00	25.00	5.60	1.90	6.00	16.00	129.00	11.60	0.34	0.58
KR-74	70.00	65.00	4.00	15.00	20.00	13.00	8.30	1.30	7.30	18.50	127.00	15.60	0.16	0.77
KR-75	65.00	59.00	4.00	12.00	25.00	25.00	7.90	1.95	4.10	18.00	129.00	12.00	0.25	0.60
KR-76	81.00	73.00	6.00	16.00	27.00	25.00	8.70	2.30	5.40	17.00	126.00	14.10	0.26	0.70
KR-77	72.00	65.00	3.00	12.00	25.00	25.00	8.50	1.40	4.90	16.90	127.00	13.40	0.16	0.67
KR-78	81.00	70.00	5.00	16.00	24.00	15.00	5.00	0.95	7.60	19.60	128.00	12.60	0.19	0.63
KR-80	66.00	53.00	2.00	13.00	20.00	10.00	1.80	0.15	0.70	15.40	128.00	2.50	0.09	0.12
KR-82	83.00	72.00	8.00	14.00	27.00	20.00	8.60	1.10	11.30	21.60	121.00	19.90	0.13	0.99
KR-87	95.00	83.00	6.00	20.00	29.00	25.00	7.90	1.60	9.00	18.50	123.00	16.90	0.20	0.00
KR-89	75.00	68.00	3.00	14.00	23.00	10.00	6.40	1.35	6.00	21.50	127.00	12.40	0.21	0.61
KR-92	86.00	67.00	4.00	16.00	24.00	25.00	6.60	1.60	5.50	18.00	126.00	12.10	0.24	0.60
KR-96	73.00	59.00	4.00	12.00	25.00	20.00	5.00	1.30	5.60	15.80	126.00	10.60	0.26	0.52
KR-98	92.00	78.00	7.00	20.00	24.00	10.00	6.10	0.90	5.90	15.50	126.00	11.90	0.15	0.59
KR-99	78.00	71.00	7.00	22.00	23.00	10.00	3.80	0.45	10.60	18.10	126.00	14.40	0.12	0.72
KR-100	96.00	79.00	6.00	20.00	29.00	15.00	9.40	1.85	4.90	16.50	119.00	14.30	0.20	0.71
KR-105	81.00	70.00	4.00	17.00	21.00	13.00	7.40	1.25	4.50	17.00	126.00	11.90	0.17	0.59
KR-113	73.00	59.00	2.00	15.00	22.00	20.00	5.90	0.90	1.90	17.80	126.00	7.70	0.15	0.38
KR-114	70.00	60.00	3.00	12.00	21.00	8.00	8.50	1.15	1.00	15.20	127.00	9.40	0.14	0.47
KR-119	65.00	61.00	6.00	16.00	21.00	18.00	8.40	1.30	5.20	18.30	129.00	13.60	0.15	0.68
KR-129	63.00	53.00	2.00	12.00	24.00	13.00	4.90	0.90	1.10	13.50	130.00	6.00	0.18	0.30
KR-130	80.00	64.00	3.00	14.00	24.00	20.00	10.00	1.80	1.80	15.20	129.00	11.80	0.18	0.58
KR-131	60.00	52.00	3.00	12.00	18.00	5.00	3.20	0.35	0.90	14.30	130.00	4.10	0.11	0.20
KR-141	48.00	43.00	3.00	11.00	21.00	8.00	2.00	0.50	0.80	12.30	128.00	2.80	0.25	0.00
KR-143	50.00	45.00	1.00	10.00	22.00	8.00	2.40	0.50	0.50	19.50	126.00	2.90	0.21	0.00
KR-145	50.00	43.00	3.00	11.00	22.00	10.00	3.90	0.85	0.70	13.00	124.00	4.60	0.22	0.00
KR-147	57.00	42.00	2.00	12.00	15.00	5.00	1.80	0.20	0.80	14.50	124.00	2.60	0.11	0.00
KR-152	75.00	64.00	6.00	16.00	25.00	25.00	6.00	2.30	2.80	18.10	126.00	8.80	0.38	0.43
KR-155	61.00	57.00	6.00	16.00	25.00	20.00	6.00	1.05	4.40	16.50	126.00	10.40	0.18	0.52
KR-157	76.00	60.00	4.00	16.00	26.00	15.00	4.40	0.65	1.30	15.30	128.00	5.70	0.15	0.28
KR-164	63.00	59.00	3.00	16.00	23.00	10.00	3.80	0.85	1.00	15.90	126.00	4.80	0.22	0.23
KR-167	73.00	62.00	3.00	14.00	28.00	15.00	11.60	1.95	0.70	13.80	124.00	12.20	0.17	0.61
KR-168	68.00	61.00	5.00	15.00	24.00	15.00	8.20	1.30	3.90	15.00	124.00	12.10	0.16	0.60
KR-170	63.00	59.00	3.00	15.00	26.00	15.00	6.30	1.35	1.20	17.30	120.00	7.50	0.21	0.37
KR-174	73.00	57.00	4.00	14.00	23.00	18.00	10.10	1.20	4.20	15.00	129.00	14.30	0.12	0.00
KR-177	66.00	60.00	5.00	13.00	26.00	15.00	9.50	1.75	3.20	16.20	126.00	12.70	0.18	0.63
KR-182	75.00	69.00	4.00	14.00	28.00	30.00	11.70	2.45	5.60	18.30	131.00	17.30	0.21	0.86

KR-183	67.00	59.00	4.00	14.00	18.00	10.00	8.90	1.30	3.50	20.50	129.00	12.40	0.15	0.62
KR-184	78.00	69.00	7.00	18.00	30.00	25.00	10.00	2.55	11.30	15.70	130.00	21.30	0.26	0.00
KR-186	78.00	65.00	4.00	15.00	24.00	18.00	8.50	1.10	4.50	23.00	127.00	13.00	0.13	0.00
KR-189	65.00	60.00	4.00	14.00	23.00	8.00	7.50	1.20	9.50	22.00	128.00	17.00	0.16	0.85
KR-190	79.00	72.00	4.00	15.00	22.00	15.00	11.00	1.60	6.90	19.60	120.00	17.90	0.15	0.89
KR-196	92.00	89.00	5.00	20.00	28.00	30.00	13.00	3.90	8.10	18.00	122.00	21.10	0.30	0.00
KR-197	78.00	75.00	6.00	16.00	26.00	18.00	11.00	1.90	7.80	16.50	126.00	18.80	0.17	0.94
KR-198	74.00	70.00	5.00	15.00	22.00	18.00	8.50	6.45	4.10	18.70	127.00	12.60	0.76	0.62
KR-199	86.00	67.00	4.00	15.00	27.00	15.00	8.10	1.25	2.90	17.60	128.00	11.00	0.16	0.54
KR-200	74.00	60.00	3.00	16.00	26.00	10.00	6.20	1.00	4.10	17.60	126.00	10.30	0.16	0.51
KR-201	77.00	74.00	5.00	17.00	33.00	25.00	12.10	3.80	5.70	17.50	122.00	17.70	0.32	0.88
KR-202	84.00	71.00	3.00	17.00	28.00	20.00	9.10	1.95	4.20	18.70	122.00	13.30	0.22	0.66
KR-203	91.00	87.00	7.00	19.00	29.00	28.00	10.70	1.65	11.50	17.90	121.00	22.20	0.15	1.10
KR-204	86.00	82.00	4.00	17.00	24.00	18.00	9.80	1.80	9.30	21.00	122.00	19.10	0.18	0.95
KR-207	74.00	70.00	4.00	16.00	21.00	15.00	8.50	1.55	5.80	17.20	121.00	14.20	0.18	0.71
KR-211	68.00	61.00	6.00	16.00	28.00	20.00	7.50	1.25	2.00	16.50	127.00	9.40	0.17	0.47
KR-213	65.00	56.00	2.00	11.00	23.00	8.00	2.70	0.55	2.30	27.20	121.00	4.90	0.21	0.00
KR-215	66.00	54.00	5.00	14.00	22.00	8.00	1.10	0.65	3.20	18.50	121.00	4.30	0.59	0.21
KR-217	55.00	37.00	2.00	10.00	14.00	5.00	1.40	0.10	0.70	14.50	123.00	2.10	0.07	0.10
KR-223	83.00	69.00	6.00	17.00	27.00	25.00	5.30	0.85	7.20	21.40	122.00	12.50	0.16	0.62
KR-225	81.00	74.00	8.00	16.00	27.00	13.00	10.50	1.35	1.30	15.30	127.00	11.70	0.13	0.58
KR-241	60.00	56.00	4.00	15.00	23.00	8.00	3.20	0.55	2.40	16.50	126.00	5.60	0.17	0.27
KR-242	52.00	42.00	4.00	11.00	19.00	10.00	3.90	0.80	2.10	14.90	126.00	6.00	0.21	0.29
KR-247	72.00	62.00	5.00	15.00	15.00	8.00	3.40	0.55	4.70	18.20	126.00	8.10	0.16	0.40
KR-249	62.00	57.00	3.00	15.00	20.00	13.00	5.30	2.20	1.60	11.30	130.00	6.90	0.42	0.34
KR-250	58.00	55.00	4.00	14.00	22.00	8.00	2.50	0.45	1.00	17.20	129.00	3.50	0.18	0.17
KR-252	61.00	58.00	5.00	15.00	28.00	18.00	8.60	1.85	3.40	15.60	130.00	12.00	0.22	0.59
KR-256	65.00	63.00	7.00	16.00	26.00	15.00	7.50	1.10	7.80	18.30	123.00	15.20	0.15	0.76
KR-260	72.00	62.00	6.00	13.00	21.00	10.00	7.50	1.05	4.60	18.50	129.00	12.00	0.14	0.60
KR-267	69.00	60.00	7.00	13.00	26.00	20.00	6.70	1.10	7.70	16.10	129.00	14.40	0.17	0.71
KR-268	60.00	50.00	2.00	11.00	26.00	20.00	4.40	1.05	0.10	12.80	128.00	4.50	0.24	0.22
KR-271	50.00	46.00	5.00	14.00	21.00	5.00	2.80	0.35	0.70	12.70	124.00	3.50	0.13	0.17
KR-279	74.00	68.00	6.00	17.00	24.00	15.00	7.20	0.90	5.10	18.80	126.00	12.30	0.13	0.00
KR-283	71.00	67.00	6.00	15.00	20.00	13.00	3.80	0.45	7.30	18.20	128.00	11.10	0.12	0.55
KR-295	85.00	71.00	6.00	23.00	23.00	13.00	4.00	0.70	7.70	16.50	130.00	11.70	0.18	0.58

KR-296	65.00	57.00	9.00	15.00	23.00	28.00	13.40	1.90	9.50	18.40	129.00	22.90	0.14	1.14
KR-298	73.00	79.00	6.00	18.00	31.00	30.00	13.30	2.25	7.00	20.60	124.00	20.20	0.17	1.01
KR-299	71.00	76.00	5.00	13.00	30.00	30.00	7.30	1.25	7.40	17.90	125.00	14.70	0.17	0.73
KR-300	76.00	70.00	6.00	17.00	30.00	30.00	11.00	1.60	7.40	18.30	124.00	18.40	0.15	0.91
KR-301	89.00	72.00	5.00	18.00	30.00	30.00	8.90	1.75	4.40	15.60	127.00	13.30	0.20	0.66
KR-302	88.00	84.00	6.00	16.00	25.00	30.00	11.00	2.55	8.40	15.50	126.00	19.40	0.23	0.00
KR-329	58.00	52.00	4.00	14.00	20.00	8.00	2.10	0.30	3.00	20.30	129.00	5.10	0.15	0.96

PH- Plant height, SL- shoot length, NPT- number of productive tillers, PL- Panicle length, RL- Root length, DSW- dry shoot weight, DRW- dry root weight, SY- yield per plant, DFF- days to fifty percent flowering, r/s ratio- root to shoot ratio, P/seed- Phosphorus content of seed, ISM- Improved Samba Mahsuri .

Appendix B

Overall Performance of 112 RILs along with their parents for various agro morphological and yield related traits under normal P condition

Genotypes	PH	SL	NPT	PL	RL	RV	DSW	DRW	YP	1000sw	biomass	r/s ratio
Wazuhophek	104.00	97.00	12.00	27.70	30.00	140.00	42.10	7.90	18.37	21.10	60.47	0.19
ISM	83.00	80.00	9.00	15.30	28.00	70.00	24.50	3.50	16.30	12.20	40.80	0.14
KR-2	104.00	98.00	7.70	23.30	25.50	75.00	16.60	4.25	8.60	17.10	25.20	0.26
KR-3	100.00	95.00	10.30	20.00	25.00	60.00	16.35	3.00	11.00	18.00	27.35	0.18
KR-4	107.00	100.00	8.70	23.70	25.00	65.00	20.50	3.75	8.67	21.30	29.17	0.18
KR-5	114.00	104.00	10.30	20.30	25.50	110.00	26.00	8.50	20.27	23.30	46.27	0.33
KR-6	111.00	104.00	8.30	21.30	25.00	110.00	22.75	9.95	11.17	18.40	33.92	0.44
KR-8	100.00	91.00	7.00	24.00	27.50	75.00	17.45	3.85	9.87	19.20	27.32	0.22
KR-9	104.00	84.00	7.30	20.30	25.50	80.00	21.25	5.05	6.47	21.80	27.72	0.24
KR-10	100.00	96.00	9.00	23.70	26.00	85.00	21.70	4.55	9.53	18.50	31.23	0.21
KR-11	110.00	97.00	8.70	21.70	24.00	75.00	17.65	4.50	7.33	25.50	24.98	0.26
KR-13	99.00	90.00	6.30	19.30	25.00	90.00	22.90	6.25	9.70	17.90	32.60	0.27
KR-14	110.00	102.00	10.00	19.70	26.50	95.00	20.40	5.30	10.93	17.70	31.33	0.26
KR-16	107.00	102.00	8.30	19.00	24.50	95.00	23.75	3.75	9.87	18.70	33.62	0.16
KR-18	105.00	99.00	8.30	18.00	21.50	70.00	22.30	5.00	9.67	17.70	31.97	0.22
KR-19	101.00	92.00	7.70	20.00	21.00	50.00	13.15	3.80	4.83	17.70	17.98	0.29
KR-20	112.00	104.00	8.70	22.30	24.50	110.00	20.80	9.15	10.87	19.30	31.67	0.44
KR-21	98.00	92.00	6.30	19.00	25.00	40.00	16.10	4.00	7.80	17.40	23.90	0.25
KR-23	111.00	105.00	7.30	21.00	27.00	70.00	19.80	4.75	12.00	19.70	31.80	0.24
KR-24	103.00	96.00	9.00	19.70	24.00	65.00	16.30	4.65	9.47	18.80	25.77	0.29
KR-25	112.00	107.00	6.70	21.30	25.00	70.00	19.20	4.80	7.67	18.90	26.87	0.25
KR-26	111.00	105.00	9.00	22.30	24.00	60.00	25.00	2.45	10.33	19.00	35.33	0.10
KR-27	107.00	103.00	8.00	24.70	25.50	95.00	22.80	5.75	12.83	21.80	35.63	0.25
KR-31	109.00	95.00	10.30	20.70	25.00	110.00	25.75	4.75	11.07	17.90	36.82	0.18
KR-32	102.00	96.00	9.00	20.70	25.50	70.00	22.80	5.50	13.07	17.10	35.87	0.24
KR-38	105.00	99.00	8.30	18.30	25.00	65.00	17.40	4.70	9.83	16.20	27.23	0.27
KR-39	109.00	105.50	10.30	22.70	25.00	75.00	19.10	4.25	12.03	22.30	31.13	0.22
KR-42	104.00	99.00	11.70	18.70	25.00	90.00	20.80	9.35	9.03	14.30	29.83	0.45
KR-45	116.00	111.00	13.00	19.70	28.50	105.00	25.65	6.25	9.07	17.90	34.72	0.24
KR-46	106.00	102.00	8.70	19.00	26.50	40.00	16.50	3.15	13.87	18.20	30.37	0.19
KR-49	113.00	108.00	11.00	19.00	28.00	55.00	20.10	3.15	10.83	18.40	30.93	0.16
KR-58	105.00	103.50	6.30	21.70	24.00	70.00	20.10	3.50	9.67	17.20	29.77	0.17
KR-65	110.00	104.00	14.70	22.00	30.50	115.00	30.15	5.75	12.07	18.30	42.22	0.19
KR-67	98.00	95.00	8.30	20.70	24.00	55.00	19.85	5.15	10.83	14.90	30.68	0.26
KR-70	108.00	105.50	6.70	23.30	27.00	50.00	16.80	4.10	9.10	21.00	25.90	0.24
KR-71	117.00	106.00	7.30	22.00	26.50	55.00	18.60	4.50	9.93	22.30	28.53	0.24

KR-73	97.00	93.50	7.70	20.70	22.50	60.00	21.15	4.50	7.63	17.50	28.78	0.21
KR-74	100.00	96.00	9.30	21.00	25.00	85.00	21.85	5.75	8.50	16.00	30.35	0.26
KR-75	88.00	84.00	8.00	19.30	27.00	45.00	17.50	3.50	6.17	19.20	23.67	0.20
KR-76	108.00	100.00	8.70	19.30	26.00	65.00	18.50	3.75	6.43	18.70	24.93	0.20
KR-77	99.00	92.00	7.70	19.00	25.00	40.00	14.25	2.65	6.40	21.80	20.65	0.19
KR-78	110.00	105.00	8.70	19.70	26.00	65.00	25.50	5.25	10.10	20.50	35.60	0.21
KR-80	111.00	97.00	6.30	18.30	23.50	70.00	16.75	4.65	8.00	17.10	24.75	0.28
KR-82	136.00	119.00	7.00	21.30	30.00	105.00	26.35	5.65	18.00	22.50	44.35	0.21
KR-87	111.00	105.50	5.70	21.70	25.00	60.00	18.15	4.05	20.00	18.10	38.15	0.22
KR-89	107.00	105.50	9.30	20.70	28.00	110.00	24.00	7.50	6.90	22.20	30.90	0.31
KR-92	104.00	101.00	8.70	22.70	27.50	75.00	22.00	4.25	9.50	21.90	31.50	0.19
KR-96	96.00	90.00	8.70	22.70	25.00	75.00	20.00	5.00	6.33	13.70	26.33	0.25
KR-98	110.00	107.00	8.00	21.00	28.50	75.00	20.40	5.00	9.87	16.70	30.27	0.25
KR-99	101.00	83.50	8.70	21.00	22.00	45.00	11.95	2.95	19.00	16.80	30.95	0.25
KR-100	104.00	100.50	8.00	22.00	24.50	55.00	16.65	3.75	8.13	17.00	24.78	0.23
KR-105	108.00	103.50	9.00	24.70	28.50	105.00	23.60	6.10	5.10	16.10	28.70	0.26
KR-113	111.00	104.50	12.30	23.70	24.00	25.00	13.35	3.30	11.30	19.80	24.65	0.25
KR-114	107.00	101.50	7.30	21.30	23.00	50.00	17.65	3.60	6.37	19.40	24.02	0.20
KR-119	92.00	86.50	6.30	21.00	24.00	50.00	15.45	2.50	5.70	18.50	21.15	0.16
KR-129	112.00	108.00	8.70	17.70	26.50	65.00	28.00	4.85	10.23	15.70	38.23	0.17
KR-130	117.00	112.00	11.00	22.00	23.00	105.00	20.90	3.75	10.00	22.10	30.90	0.18
KR-131	99.00	93.00	8.00	19.70	29.00	85.00	19.50	3.75	6.47	20.50	25.97	0.19
KR-141	91.00	87.50	7.70	20.00	25.00	50.00	13.05	2.40	6.13	12.50	19.18	0.18
KR-143	115.00	110.00	8.00	20.30	26.50	60.00	17.05	3.20	6.77	20.50	23.82	0.19
KR-145	90.00	85.00	9.00	20.30	26.50	60.00	19.05	3.80	4.97	16.80	24.02	0.20
KR-147	89.00	75.00	7.70	18.00	27.50	65.00	21.35	3.60	14.47	21.00	35.82	0.17
KR-152	101.00	99.50	15.70	22.00	29.00	110.00	22.25	5.00	14.53	17.40	36.78	0.22
KR-155	97.00	92.00	7.70	20.30	27.50	75.00	15.45	4.00	7.23	18.90	22.68	0.26
KR-157	104.00	100.00	8.30	19.70	24.00	55.00	19.95	4.50	10.00	22.00	29.95	0.23
KR-164	100.00	95.00	7.70	21.30	31.50	85.00	32.25	7.00	12.43	20.60	44.68	0.22
KR-167	110.00	106.00	6.00	21.30	26.00	75.00	21.10	4.50	14.77	16.50	35.87	0.21
KR-168	104.00	99.00	8.70	20.00	21.50	60.00	14.90	3.90	5.37	14.80	20.27	0.26
KR-170	94.00	90.50	7.00	18.00	27.00	40.00	14.10	3.05	6.50	18.80	20.60	0.22
KR-174	100.00	97.00	8.00	19.00	22.50	40.00	17.70	2.50	7.07	13.20	24.77	0.14
KR-177	98.00	92.00	11.70	19.70	28.00	90.00	23.05	7.55	4.50	14.50	27.55	0.33
KR-182	100.00	97.00	6.30	19.30	26.50	90.00	23.40	4.80	8.00	22.00	31.40	0.21
KR-183	109.00	81.00	7.30	20.00	26.50	80.00	23.15	5.00	4.93	19.80	28.08	0.22
KR-184	112.00	107.00	10.30	20.30	23.00	40.00	19.10	3.25	16.00	15.80	35.10	0.17
KR-186	117.00	108.00	8.30	18.30	26.00	80.00	26.10	5.50	6.17	18.90	32.27	0.21
KR-189	106.00	101.00	9.30	21.00	24.50	50.00	15.15	3.10	18.70	20.00	33.85	0.20

KR-190	105.00	102.00	6.30	20.30	24.50	55.00	18.05	3.50	11.27	19.10	29.32	0.19
KR-196	105.00	101.00	8.00	18.30	26.00	65.00	18.95	3.15	9.17	18.30	28.12	0.17
KR-197	111.00	105.00	8.00	17.30	25.00	90.00	18.20	4.50	10.53	18.00	28.73	0.25
KR-198	103.00	100.00	9.70	19.30	24.00	35.00	16.10	2.50	6.63	18.00	22.73	0.16
KR-199	110.00	104.00	9.00	20.70	26.00	105.00	23.75	5.85	7.63	19.50	31.38	0.25
KR-200	109.00	86.00	7.00	22.30	26.00	65.00	19.50	4.00	5.10	19.50	24.60	0.21
KR-201	98.00	94.00	10.30	20.30	26.50	75.00	14.20	4.65	6.77	18.20	20.97	0.33
KR-202	107.00	102.00	8.70	18.30	23.00	95.00	24.60	6.00	4.93	15.50	29.53	0.24
KR-203	116.00	107.50	10.70	20.70	25.50	95.00	23.05	4.60	15.00	18.80	38.05	0.20
KR-204	105.00	102.00	6.30	22.70	21.00	20.00	14.55	5.90	13.00	19.90	27.55	0.41
KR-207	98.00	70.00	9.00	22.30	18.50	20.00	13.90	2.05	7.03	13.60	20.93	0.15
KR-211	107.00	103.00	10.30	20.70	23.50	70.00	15.25	2.75	10.50	16.90	25.75	0.18
KR-213	150.00	137.00	10.30	23.70	26.50	80.00	32.25	5.00	17.77	18.00	50.02	0.16
KR-215	107.00	105.00	9.30	21.30	23.00	85.00	18.50	2.75	3.63	13.20	22.13	0.15
KR-217	100.00	95.00	8.30	18.70	25.50	60.00	20.25	5.45	6.23	20.30	26.48	0.27
KR-223	119.00	112.50	11.00	21.70	22.00	85.00	20.15	4.00	14.20	23.40	34.35	0.20
KR-225	104.00	99.00	10.30	20.30	21.00	75.00	18.80	3.75	7.50	15.90	26.30	0.20
KR-241	99.00	95.00	9.70	25.30	21.50	75.00	27.50	5.50	15.93	19.30	43.43	0.20
KR-242	93.00	87.00	18.30	18.70	31.50	100.00	28.90	6.50	13.20	18.10	42.10	0.22
KR-247	108.00	104.00	6.00	24.00	28.00	75.00	23.00	7.00	7.67	18.60	30.67	0.30
KR-249	97.00	95.50	7.70	22.30	24.50	55.00	21.25	5.30	10.00	14.40	31.25	0.25
KR-250	109.00	100.00	9.30	19.30	23.50	70.00	24.60	5.10	10.43	19.70	35.03	0.21
KR-252	100.00	94.00	10.70	22.00	22.50	55.00	22.10	4.10	4.70	13.50	26.80	0.19
KR-256	101.00	99.50	8.30	21.30	27.50	65.00	17.75	5.00	10.53	20.50	28.28	0.28
KR-260	97.00	93.00	9.30	18.30	22.50	65.00	15.65	3.70	7.00	7.00	22.65	0.24
KR-267	87.00	84.00	8.00	20.70	20.50	40.00	18.10	5.25	8.50	17.30	26.60	0.29
KR-268	99.00	95.50	8.30	20.70	28.50	55.00	20.40	3.90	7.33	17.20	27.73	0.19
KR-271	88.00	84.00	7.30	21.00	24.00	80.00	14.10	3.00	7.87	17.10	21.97	0.21
KR-279	106.00	96.00	10.00	21.30	25.50	55.00	26.20	4.00	7.40	18.70	33.60	0.15
KR-283	101.00	97.00	7.70	22.70	21.00	40.00	16.25	3.40	8.90	17.70	25.15	0.21
KR-295	110.00	113.50	9.70	22.00	26.00	50.00	23.30	4.25	8.60	15.50	31.90	0.18
KR-296	77.00	72.00	10.00	22.30	22.00	35.00	11.50	2.45	18.30	18.80	29.80	0.21
KR-298	103.00	98.00	8.70	20.70	22.00	95.00	22.80	3.90	11.23	19.90	34.03	0.17
KR-299	109.00	106.00	8.00	22.70	26.50	40.00	18.85	4.50	11.57	18.40	30.42	0.24
KR-300	104.00	100.00	8.00	20.30	22.50	30.00	21.40	3.25	9.33	16.60	30.73	0.15
KR-301	96.00	89.50	11.00	22.70	21.00	40.00	17.85	3.20	6.20	16.90	24.05	0.18
KR-302	111.00	104.50	8.70	19.30	25.50	45.00	22.50	4.40	9.70	15.80	32.20	0.20
KR-329	110.00	105.00	10.00	21.70	22.50	50.00	18.25	3.00	15.47	23.80	33.72	0.16

PH- Plant height, SL- shoot length, NPT- number of productive tillers, PL- Panicle length, RL- Root length, RV- Root volume, DSW- dry shoot weight, DRW- dry root weight, YP- yield per plant, 1000sw- 1000 grain weight, biomass- total biomass, r/s ratio- root to shoot ratio

Appendix C

List of SSR markers used for parental polymorphism survey for QTL analysis

SI. NO	Primer name	Chr. no	Forward Primer	Reverse Primer	Position (Mb)
1	RM10695	1	CCTTCGACTCCATGAAACAAACG	TCTCTTTGCCCTAACCCCTATGTCC	10.98
2	HRM11099	1	CTAGGTCTGTACACGCACTGAAGG	CAAATAGCTCGACACAGGAGATCG	20.05
3	RM10861	1	GATCGAGGAATATGATCCAACCTCG	ATTTCTGGTGTGAGCACTGATCG	14.19
4	RM8047	1	TGCATGTAGGAGCTAAGTAAGG	GATTTGGAGCCAGTAGTTGG	0.29
5	RM10033	1	AGAGTGCGTAATAGCTCACTGATCG	ACCCAGGACGAACAAATCTAGGC	0.52
6	RM10139	1	CAACGAGACCTCCGTTAGAGC	CCAAGAGATAGTGGGTGTATGATAGG	2.78
7	RM10167	1	GTGCCTAAGATTACAGCGCTGTGG	GCGGGACATTCATGTACACATTGC	3.39
8	RM10247	1	ATGCATTTCGAGCCCATTCTAGC	CGGTTGTGGGTACGTATGCTTCG	4.22
9	RM3148	1	GCTTTGGTATTTGCAGGTTACG	CTATTGCTCGAACACTTTGCTTCTCC	0.74
10	RM10051	1	AGTACCGTATTGCAAGAGAAGC	GGTTTGGTTCGATTTAGTCTCC	0.92
11	RM10078	1	ATTGACGGTAGTGGCATGAGAGG	CCGTGTTATTTAGTGTGCGTTGG	1.41
12	RM10133	1	TGACAGGTCACCTTCTCACAGC	AGACTGGACACAGCAACTCTCC	2.6
13	RM6902	1	CACAAACCGAAAGTTGCCCTTCC	TTCGGAGAAGACCGAGACGTAGATGC	3.8
14	RM1329	1	CGGAGCTCAATCGAATCTAGACC	TTCCTCATATCCTCCAGCTCTTAGC	3.94
15	RM10115	1	ACAAGACGAGGTAACACGCAAGC	GCGAAGGATCAACGATGATATGG	2.15
16	RM8105, RM3426	1	CCGATGATGGAAGCACAGATAGG	TTCTCATTCTCGAAGGCTTACGG	4.05
17	RM10655	1	AGTACCGTTGAATCCGATATGC	TGGTTGAGGTGCTGAATTGG	10.36
18	RM10662	1	GATCTCAACACACATGCAAACCTGG	CTAGCGTATGCAAGGAAGGATGC	10.47
19	RM10702	1	ACGATAAATCAAGCGGCTACTCG	CTTTCAGCAGCACCTTCTCAGG	11.05
20	RM11002	1	GCAAAGGACAACGACTCATACAGAGG	TCCGGGTGAAATGCTACAACG	17.8
21	RM11033	1	TGTTTCAGAGTTCAGAGTCACACG	AGGACAAGCCCACTTATTGAACC	18.67
22	HRM11449	1	AAGTCGACAATGTCATCTCAGG	TTCAGCAATAAGCTTCGCTACC	26.81
23	RM11111	1	CTGTTGTTCGGTTACACCTTGTGC	CCACTCCTCTATAGCGTCATTACCC	20.39
24	HRM11997	1	TGTTGTACCAGATGCCCATGTACC	CCGCGTTCACATAACGATGC	38.68
25	RM10936	1	ACGGTTTGAAGTGTTCTGATAGG	TGGTACTGCATAATCTCAGCATCG	15.73
26	RM10963	1	TCCAGACTGTGACGAGCAGTACC	ATAACCAGGATCTACCCACATACCC	16.29
27	RM10066	1	TCGCCATCTCAATCCAATCTAGG	GTCTGAAGCATAGGTTTGTCTGAAGC	1.31
28	RM10092	1	TTGACTCAGCTGAAACGATGACC	CAGGGTTAGACGGGAGCTTCG	1.66
29	RM4959	1	GATCGGATCCTTCCAAATTGC	TATTTCTCGCTAGCTCCAACC	0.45
30	RM10060	1	GAGATGCGTGCAGTGACAAGG	CCAGAATAAATGCCATGCAGTCG	1.21
31	RM10106	1	CAGCAAGCTGATCTGCAACTCC	CAGATACCAACTTTACTTGCCACTCC	1.84
32	RM10109	1	GAGACCATCGATTTGCGGTGAGC	ACGGTACATCAGCCAAGCAAGAAAGG	1.98
33	RM10110	1	TGTCACAGCAGCTGGTAATACTTGG	GCAACAGCAGCAATCATACATGG	2.08
34	RM10123	1	ACACATCCCTCATCATCATCTCC	GAATCCGATCTTGTTCTGATACCC	2.28
35	RM11024	1	ATACACCTAGAGGCCTGGACACC	TGCTCGTTGAACTGTGACGTACC	18.47

36	RM10720	1	GCAAACGTCTACGTGAGAAACAAGC	GCATGTGGTGCCTTAACATTTGG	11.39
37	RM495	1	ATGATGATGGACGACGACAACG	TGAATCCAAGGTGCAGAGATGG	0.21
38	WR1.7	1	CGCACACGTTTGTGTGAATTG	CGCTTATCTACGGATGGATG	1.7
39	RM493	1	GTACGTAAACGCGGAAGGTGACG	CGACGTACGAGATGCCGATCC	12.26
40	RM10190	1	CCCAGCGACTGACATCGAACC	TTATCTTACACGGCTCTGTGATGTGC	3.76
41	RM10009	1	GATGCTCCGGAATAACTAGATTGG	GGAATTACAGCTGTCTTGGAAGG	0.18
42	RM8069	1	CGTTCAAAGCGAGCTTAATTGC	CTACGGCGGCTAAACATAACTCC	1.56
43	RM10153	1	GCGACCGAATAAATTTCCAAGG	AAATACGAGAGCCCTTCCATCC	3.1
44	RM3652 (RM10174)	1	TGAGGAGGTGGCGGAAGAGG	CTCTGATGCGTCCGAAGAGAGG	3.57
45	RM10106	1	CAGCAAGCTGATCTGCAACTCC	CAGATACCAACTTTACTTGCCACTCC	1.84
46	RM10147	1	GAGAGGAACGAGAAAGAGGATAAGC	CCCTATCTTTACCCTAGTCGATCTCC	2.98
47	RM10149	1	AGAAGCCCGTGGAGTTTGTGG	AAACGAGCACTCGCGGAAGC	3.03
48	RM10162	1	GGGCATGTCAATTCCTACTCCTACCG	CCACAAAGTCGCAATGTCTGTAGC	3.28
49	RM10255	1	GATTCACACATAGAGTGAATGTCG	CTTTGCCAGGAGAGTTCTAACG	4.35
50	RM3443	2	CCGCATCTGCACCTCTAAATCC	GTACACGCCTGTAGCTCGTTGC	14.43
51	HRM13238	2	CGAACTCCCAATTACAGATCACG	TCATCCAAGACTTAGGTGGAGAAGC	17.54
52	RM13534	2	AAGGAGTTGGGTGCTGTGAAGG	TACTTCTTTATAGCCCCGCTCGTTGC	22.77
53	RM12434	2	TTGGTCATCTTTGGTTGGTTCAGG	GGACAATTCACACAGCAGAATTGG	2.07
54	RM13045	2	AGTAGCCAACACATCGTCCATCG	CGTCCTCATGTATTAGGGTGAGAGC	11.89
55	RM6942	2	CGAACTCCCAATTACAGATCACG	TCATCCAAGACTTAGGTGGAGAAGC	17.54
56	RM13581	2	TAATCAAACATCGGCACAGTGG	GCGTTGCGACACTAAACAATGC	23.86
57	RM6938	2	CCGATTAGCGATTGATATGGAGTAGG	AGTGCACAGCCATGGAATTATGC	0.97
58	RM13070	2	CCACCGGAGATCATAGGTTCTG	AAGTTAGCCCTCAACCGCTATGG	12.61
59	RM154	2	GACGGTGACGCACTTTATGAACC	CGATCTGCGAGAAACCCTCTCC	1.08
60	RM452	2	GTGGACTTGCGGAGATGCTACG	GTTAAGGGCAGCCACCAGATCG	9.56
61	RM300	2	GGGCTTAAGGACTTCTGCGAACC	AGCGATCCACATCATCAAATCG	13.19
62	HRM13154	2	GGTACTTAGCGTGCACTTTAACC	TAGGTAAGTACGAGAGCGATAGAGG	15.32
63	HRM13155	2	TACCAACAGGGAGTTGTCTCTCG	AGCGACGGTGTAAGAATAAGTCG	15.36
64	RM1063	2	TTGATCCACTATCGTGGCCTACC	TGGCAAGTGCAACTGCAAGG	35.12
65	RM5862	2	CCTCCTGAAGGGTAAAGGATTGG	TCCACACATGATCGCTACATCG	6
66	RM13155	2	TACCAACAGGGAGTTGTCTCTCG	AGCGACGGTGTAAGAATAAGTCG	15.36
67	RM1385	2	GACAGGTAAGGTGTGGTGTAAGG	AAACCTTTCTCAAACGCACACG	26.66
68	RM13761	2	ATCCATCCTCAGACACACATGG	GAATTCCTGATATTCTCGCGTAGC	27.52
69	RM14140	2	CCTCCCTCTCCAAACACATTGC	TCATCAGCAGACAGAATGTTGACC	34.41
70	RM12398	2	CCTACTACTGTTTGCCGGTTTGC	GCAATAAAGTGGAAGCCTATCTCG	1.64
71	RM12678	2	AGGAGAGCAGCCCACTACTCCTATGG	TCTGAGAGGCCTACCCGCATGG	5.76
72	RM12859	2	AACCATGATGGCTATGCTATGC	TCCTGATCTCCTGTACATGTATGC	8.64
73	RM12982	2	CAGTAACCTCGAGCACCATTAGG	TCGGACGAATTACCAAACTACCC	10.4
74	RM5179	2	TCCCAAACACAATCCTTTGACC	ACACGATTAACCTTCGTGAGCTACC	13.98
75	RM13429	2	ACTCACACCTATGTGTCCGTTCC	AAGGAGTGCTCTAGAAATCGTGACC	21.16

76	RM13604	2	ACTGCAGGTGGTACCTTGATCC	GTGTTGTTCACTTGTTCAGCTTCC	24.57
77	RM13962	2	ACGACGGAAACACAATTCCTGG	GAGCCATATTTAGTCCCGTTGG	31.36
78	RM1063	2	TTGATCCACTATCGTGGCTACC	TGGCAAGTGCAACTGCAAGG	35.12
79	CHR2-28.2	2	CATGCACTGACGTAATGCAGACC	TCTGACATGAGCTGTGGAGAAGG	28.2
80	RM2578	2	TCCAGTTGGGCCAGGTGACG	ACTTGGTGGCTGTCATTGGTTGG	32.27
81	RM6367	2	GCAACCACGACATCAAAGAAACC	GGAGGTTAGTGCTTCGGAGTGG	1.13
82	RM12390	2	GAGAATCGCGGAAGGGAAGC	AAGCAGAGAGCAAAGCGAAAGG	1.57
83	RM6151	2	ATCCACCGGATTTCTCCTACTCC	CCATTTCGAGCCCAAACATAACC	1.94
84	RM12460	2	TGGCACTACAGTGACAACAAACC	AGGGACTTTATCCAAAGGACACG	2.63
85	RM5654	2	ATTACGTGGGTCGAACCAGAGC	CAAGTTCGTTACAGCAGAGGAAAGC	3.42
86	RM13263	2	AAGATTGCACACTGGTGTCTCC	AGAAGAGCCGGTCTTTGTCTCC	18.18
87	RM13709, RM5706	2	GTGGCTTGATTTCTGCAACTTCC	GCTGGTACCTACCAAGTATCATTTCCG	26.5
88	RMES2-1		ACCAAGGCAACCCATGAAT	ACCTGCGGCTTCTTCTCTT	1
89	RM13916	2	CCGGGACTAAAGATAGAGCTGACC	AACACAATTCACCGGTCTTAGC	30.13
90	RM13338	2	TTGATAGCCCCGACCTCTTCTCG	ATGGGATAGCGGGATAGTCAATCG	19.3
91	RM13630	2	GCCGCTGATCTCTTACCTGTTACGG	GTGCTTCGGCACAGGTCAATGC	25.14
92	RM13659	2	GAACAGATTCTTGCCAATGTGC	AGCGAGAAAGAACAGGAAGTGC	25.75
93	RM13209	2	GGTGATGCCCTAATCATAGTCTAAGC	AGGAGTGGCTTCTCGAAGTACTAGC	16.75
94	RM14096	2	GTGCATGAACGACATAGCAAAGG	TTCCGATCTGAATGCACACTGC	33.89
95	RM13328	2	TCGACCAGCTCCTCCAGTCG	GTCTTGACACGACCTTGAATGC	19.18
96	RM13445	2	GTGTTGTTTGGGATGCTCTTTGC	GAACTTCTCCACATCTGCACAACC	21.43
97	RM13131	2	TGGAGTGAGGGTAGGTGGGTTGG	CTCGAGAGTTGTGCCCATCATCC	14.6
98	RM14311	3	AGCTTGACTTCACACCCACTCG	GGACGACCTACCGATCAAGACG	1.14
99	RM15185	3	CTCCACCTACGGCCACTTCTCC	GCATTTGCCGTGAGATGACTACG	16.45
100	RM15326	3	TGAATCTACCGCTCTACTTGTGG	AAACAGTGCATCCTTCTTGTGG	20.6
101	RM15381	3	AAGGTAGAAGGGAAGGGAAGG	ACATCTTGGCATGTATGGATGG	21.94
102	RM14250	3	GATTACTGCCGATTCGATAGC	AAATGGGACATGTTCTCTCG	0.19
103	RM14391	3	GGGAGCAAATTTGATAGGTACGG	ATGTGACTGCTGCTGGAATTAGC	2.72
104	RM14698	3	TAGATCGTCTTTGTCTGATAGC	TAGTCACGAAGCTATGTGTTGC	8.43
105	RM14735	3	AATTAAGTAGTGAGCACCCGATGG	CGGTCACAAAGTGCCAGTACC	9.32
106	RM14981	3	GGCGAGCAGAAGTATAATCCAGAAGG	CGCTTGTGGCTTACTGGCTTGG	13.72
107	RM15004	3	GCCCCGTTTCCCTTATTAAGTGG	TGGATGATAAGGGAGGAACAGAGG	14.03
108	RM15151	3	GGTTCGTGCTCTTCATGTCTTGG	AGGCAAGCACTTCGAACATAAGG	15.84
109	RM15203	3	ATGGTGAGACGACAGAACAGATGC	GACGCGCTTTGTGTTTCTTGG	16.72
110	RM15404	3	TTTGCAAGGCTATCTTCTCTGG	GGAGAGCCGAAACTATTTGATTGG	22.29
111	RM15630	3	AACTCGAAGGATCTCGCCCAACC	ACCCACCTCCTCAGCTGTACG	25.88
112	RM16238	3	GCAGCGCATCATTGTATTAAGG	GGACACTAAATCAGAAACCCATGC	36.06
113	RM14931	3	GCTTCACGCACATATTGCCTTCC	ATCCTCCCACTCCCAAATTATCTCC	12.88
114	RM6759	3	CCCAGTCTTCATAGAGATATTCC	ATCCCTAGCTAGCCTTCCTTCC	32.01
115	RM489	3	GAACAGGGACACAATGATGAGG	GACGATCGGACACCTAATTACAGC	4.31

116	RM15188	3	AGCCTCCTAGAACGGCAAAGG	CTATGTTTCGTGCAACCAAGTCC	16.49
117	RM15187	3	AGCCTCCTAGAACGGCAAAGG	CTATGTTTCGTGCAACCAAGTCC	16.49
118	RM15331	3	GGTTCGGTGCTTTCTCTTCAGC	AGCGATGCCGTCCTTACACC	20.78
119	HRM15337	3	CTTTCGGGAGATGGTGTTCG	CTCCTTCCATTCCCTTCCATAGG	20.83
120	HRM15679	3	TAGATGTATGAGTCGGAATGGAGTCG	CAGACGCAGTGTGTATGAAGTTCC	26.87
121	HRM15855	3	GGAGTTTAGAAATATGGGCTCTGG	TGGTTGATGTCTGAACCGTATAGC	29.61
122	HRM16006	3	CCCGAGTCTTCATAGAGATATTCC	ATCCCTAGCTAGCCTTCCTTCC	32.01
123	RM16171	3	TTGAATCTTGGAGCCCATACTCC	TGGGCGAATTTGTGTATTGC	35.08
124	RM3029	3	GACGAACACACAGTGACATGAGG	GCCACGACTCCTGATCATATTCC	6.67
125	RM14860	3	GGAAGGTGATTTTCATCCGGTAGC	TGGCATGTTAATGCTGGTTCG	11.62
126	RM15288	3	GAGAGGCTCCTACTGCGGTTGC	CCTTCCATCTCCTTGTTCCTCTGC	18.94
127	RM15580	3	TTGGTGTCATGAACTTGTCTGG	TTGAATCTTGGAGCCCATACTCC	24.98
128	RM15831	3	AACACAATTCACCGGTCCTTAGC	ACGTGGGTGATCGTGTCTGC	29.14
129	RM16153	3	TGGTTGTGGTATAGCACGGTAAGC	TGACCCAAGGAGATACTAGGTTGC	34.8
130	RM16221	3	ATGAGGGACGACAGACGAAACG	GAAACAGGCACTCGTGAGTTTGC	35.96
131	RM14562	3	TTGCCTGAAGAGAGACTAGACATGG	GCATGGTTTGACAGTTAAGATAGGG	5.6
132	RM15251	3	CCTTACATTGGGATGCTTTAGTCC	CATCTCCCACCCTGTTGTCTG	17.71
133	RM15302	3	CAAGCTAATCATGACCGATCAACC	GTGATATATCCAATAGGGACCCATGC	19.67
134	RM15331	3	GGTTCGGTGCTTTCTCTTCAGC	AGCGATGCCGTCCTTACACC	20.78
135	RM3049	3	TTTCACCCGAGAGTATTCCAAGG	GGCATCGCATAGAGGGTATTAGC	26.61
136	RM15981	3	GGTTAAAGGGAGGACACCACTCG	TCTAGCCAGGCATGACAAGAACC	31.64
137	RM1373, RM422	3	TGCTATACCCAAATGTCCAAGC	ATCTTCTGAGTGCTGCCAAGC	33.5
138	RM15320	3	GAAGTACTTTGGCACGTCAGC	GGGACGGATGTAGTATTAGTTAGAGC	20.24
139	JGT3-26.8	3	GAGCGTTTGTAGTAAGTTTCATGGAC	GGCCCAACCCAAACACAAAT	26.8
140	RM15379	3	CCAAGAAAGCACAAACAGAAGAGATGC	TTCACAGTGGAAATTCGCTTGG	21.85
141	RM14320	3	CACCTGTAAATTAGGACACTGG	CAGTGTACTTTGAACTGCCTAGC	1.32
142	RM15497	3	CAACTCGATCCATTAAGTGC	ATTCTCATATGCTGCACACC	23.77
143	RM15930	3	CCGGTTGGTAACACTAAAGATCG	TCCCAAGGGTGAATTCTACTACG	30.76
144	RM16171	3	TTGAATCTTGGAGCCCATACTCC	TGGGCGAATTTGTGTATTGC	35.08
145	RM16582	4	AAGGCACAAAGGAACAGTGC	TTTCCATACACCCAAAGTCG	10.93
146	RM16769	4	GCCTTTGCATGGTTACACATAGG	CAAGTCTACAAGATAGGTGGATCAGC	17.47
147	RM16773	4	CCCTCCCTCAACCCAACTTCC	TGTGAGCTCTGCCTCAGTCACC	17.68
148	RM16787	4	GCATAGGAGCGTTTATAGGAACACG	TTTCGGGATCTCTGTTTCAACC	18
149	RM16791	4	GGCTGCTTCAGAGCGTTTGTAGG	AAATACGCCATCAAGTTGCACACC	18.13
150	RM16816	4	AACCACCACAGCCTCGCTTTGC	TTCCACCTCTTCTCACGTCGTTGG	18.54
151	RM16855	4	GCGAGCCTAGTTCTGATTCTAGC	TGGAGTACGTATGTTGGGTATGC	19.22
152	RM16915	4	AGATGGCTGGCCAACAGACTGG	ACAATGCCCATCCACTGCTTCC	19.9
153	RM6679	4	TTAGGCCGTAAGAGCGAACATGG	ATATGCCGATGCAGAACAAGATCG	19.91
154	RM16606	4	TGCACTTCTTTAGAGTAGGAGGAAGC	CATGCATGTGTCCAAAGATTTCG	11.62
155	RM16913	4	GTGTACGTGTTGGCTCTCTGTACG	GATGTTGCTTGTGCTGCAACC	19.82

156	RM17127	4	CTCAATGTTTCCCACAGTTACCG	TGTGTTATGTGTGCGTGATGAGC	23.52
157	RM16404	4	GTCGACCGTGCCAGATTTC	GCATCACGTGGGCTACCTTC	3.65
158	RM16495	4	TTTCTACCGCCAACCATTGC	CACCGCACGTGTATTGTTATATCC	6.46
159	RM16513	4	TGGAGTATGTTTGGATGGAAGC	TTTCTAGGTGTTCCCAACTCTCC	7.12
160	RM16569	4	ATAGACTTCACCCGGTACAACG	CTGGTATCGACATCGGCTAGG	9.72
161	RM16622	4	TTCTGAGCTAGCGACTACTACTACG	TAACCGGGACTAAAGATCAAGC	12.47
162	RM16741	4	GACGACCACGATAGTTCAGG	CCCAACAGGCATTACATACG	16.73
163	RM16801	4	CGTTCAAGGAGCTTGTGTTGATCC	GGACCGATTAAAGTGAACGTTGATGG	18.34
164	RM17377	4	ATATTACTTCGACGCTGGATCAGG	GTCAGTTCGTCAGGCACAACG	29.12
165	RM6748	4	AGAGAAGCAGCTGGTGATTAGCC	CAACGATGTACCAGTTGAATACCC	30.9
166	RM5511	4	GAGTTCGTCCCTGACAAACAGAAACG	GTGAGCGAGCGAGTGAGTGAGC	31.14
167	RM16868	4	ATATTAGAGCATCTCCGACAGC	AATCTTGGAGCCCATACTCC	19.47
168	RM16543	4	CACGATTAACCATCGTGAGCTACC	ATCGGGTGGTGAGCCTTTCC	8.32
169	RM16705	4	GGTTAAGCACTAATGGTGGTAGG	TAAGTAGTCATCTCTGCTGCAACC	15.25
170	RM16712	4	CTTTAGTCCCGGTTGGTGTACC	AACACAATTCACCGGTCCTTAGC	15.42
171	RM16738	4	AAACGGAGTGGTCCATTAGC	GAAAGGTAGATGGGTTTGTGG	16.61
172	RM16760	4	GTCCTCCCAACAACGCTCTCC	ACCCTGTAAACGGCTGAGTCC	17.11
173	RM16795	4	ATAGCATGATGCCAGGATATGG	CCCAGATCTCCTCTTCTGTTTCC	18.24
174	RM16854	4	TCGTAGATCGACTCGTAGTCGTAGG	AGAGAGGGAGTATAACGGAATGACG	19.22
175	RM16877	4	ATGTGGCACTGATTTCTCTTCC	CCCTACTACACCTGTTTGTGG	19.5
176	RM16592	4	CTTAGCACGGACACTCATATTGG	CACAATACGTTTGATGGCTTGC	11.36
177	RM17201	4	GATCGTTGCTGCTTTCAATGAGG	AGTGTTTCACCTTGGACCCATGC	25.19
178	RM16649	4	CTCCCTTCATGCGTAAGCTCTCC	GCAAACAGGATCCTCCACAAAGG	13.62
179	RM16373	4	AAATCGTTGTAGTCCCGGTTCCG	CAATTTGTAACGTCGGTCTTCC	2.69
180	RM17263	4	GGCTCCCAAGTGTAGTATACGG	TCTGTAATCGACTGATGTCTCG	26.28
181	RM17600	4	CCTCGAAATGAATTGCAGTCGAACG	GTCTTGTGCCTTGTGCCGATGG	33.81
182	RM16294	4	CTCTAGCGATCTTCTCAATCCTTGC	GAGCAGGTGAACCGACATTATTACC	0.91
183	RM16543	4	CACGATTAACCATCGTGAGCTACC	ATCGGGTGGTGAGCCTTTCC	8.32
184	RM16691	4	ACACCAACCGGGACTAAAGATCG	CACCGGTCCTTAGCTTCTGAGC	14.79
185	RM16780	4	AACACAATTCACCGGTCCTTAGC	CCGGGATTAAAGATAGAGCTGACC	17.84
186	RM17019	4	AGCACACACGACTCTACCACAGC	GGGCCATGTAACAGACATACTACTCC	21.69
187	RM3524	4	CTGTCTCCGTCTTCCTCACTCG	TGGAGAAATCTCCCTTCTGAGC	22.86
188	RM1388	4	GATCGTTGCTGCTTTCAATGAGG	AGTGTTTCACCTTGGACCCATGC	25.19
189	RM17345	4	TTTAGCTGTTTCGCACACAG	AAGGACATCCTTCATCCTCTTGC	28.37
190	RM6909, RM5709	4	TTTGGGATAAATGGGAGAGG	TCAGCTGGTTATTATGGAGAGG	32.09
191	RM2799	4	CGCTCAGTTCACGAAACCAACC	TCTCTGCACTCTGCATTACACACG	34.35
192	RM17686	4	GAACGAAGTGAACAAGCCAATCC	CCCATTACGGCTTAGGCTCAGG	35.31
193	RM16601	4	CAGGCACCACGTTTCAGGTTGG	GCATTATGCTCAAGAAAGGCTGACG	11.46
194	RM17486	4	TGGAATCACAAACCACGACTAGG	CTACCTCAAGCTCCACGACTTCC	31.29
195	RM5979	4	GTTTGAACCTCTGGTCTCAATGC	ACAGTATACGTGGCTCAATCAGG	20.78

196	RM18353	5	AGATCTCACTATTGAGTAGCCCATGC	CACCTTGCCCTTAAATACCAACC	13.87
197	HRM18857	5	ACCGGTCCTTAGCTTCTGAGC	ACCAACCGGGACTAAAGATCG	23.08
198	RM18222	5	TGATTCTCTATATGCAGCCTTGG	TATCGTGGTTTCATCGTGTGTGC	10.02
199	RM18362	5	TTACCAACCGGGACTAAAGATCG	CACCGGTCCTTAGCTTCTGAGC	14.08
200	RM18516	5	CTTTCGTCCTGTACGTAAACC	TCAAACCTACCCTCACATTCTCC	17.35
201	RM5592 (RM18799)	5	CTCGCTCTTACAACCTTTCAAGC	CACCTACCTCCACTTCTCAACC	21.97
202	RM17858	5	CACATGGTTTGTCTAGCCGATTAGG	TTCACGATGATGCTAGTAGGTGTCTG	2.06
203	RM459	5	CCTCCAGTATCGATCACCAAAGC	CGAAAGAAAAGTCATAGCACGATGG	20.09
204	RM17836	5	TTCGGCTTCCAATGTTCAATCC	ATATCGTGGCTTTCGCTGACG	1.68
205	RM18941	5	GTGAAGTGCAGCCGAAGAGC	ATCGATCTCTCATCACGATCAACC	24.64
206	RM18983	5	GTGTTTGCGGGTGTTAATCTGG	TTACTTACCAGAAGCCACACAAGG	25.49
207	RM17780	5	GGCTGATCTACACCGTCTATTGG	TATATTGCGGCCGTAGTTAGG	0.77
208	RM17920	5	TGAAGCTTCTTGTTCACTGACTCC	GATCTTATGAACCTTCGCACAACG	3.01
209	RM18178	5	TCGTTATCTTTAGTCCCGTTGG	GAGTACTACTACGTCGAAATCGTTGC	8.68
210	RM18303	5	AGCGCTCAATGTAATGACAAGC	GAAATCTGCTCTTCATGTTGC	12.74
211	RM8039	5	GCGTATGGATGGAACATGTACG	CCACCCAGCCCTGTAGATCG	13.29
212	RM18600	5	ACGAATCTGGAGAGAAGCATCCAACC	CTGCAATTTGCGACAAGGGTTCC	18.93
213	RM1386	5	TCAAGCTGCATTAGGAAGACACC	AACCTAGCTGAAACGCAACACG	19.91
214	RM3870	5	GGAGTAGATGTAAAGCCAAAGGATGC	CATGTCTGAGTATGACGGAGTATTGC	22.81
215	RM19075	5	CCTTCTCGTCATTCTTGCTCTCC	ACGAGTCTCAGTAAGCACATCAGC	27.2
216	RM19147	5	GACTCACGGAACAGTACTAAGGTTGG	CACAATCCATGCACGTAAACTCG	28.47
217	RM592	5	ACCTCACCCGAATTACTGTGATATGC	GTTGAATTGCACGCGACTCTGG	2.73
218	RM18273	5	GCACTGGTTGTTCTGCACTTAGC	GCTCTAGCTACTTGCATGGCTTAGG	11.65
219	RM17712	5	CCTCGATTTCTTCGTCCTCTGC	CCGGCTATTCTTCCTTAATACTCC	0.029
220	RM17747	5	AGGAACGTGCTCAATCGGAAGC	AATCCTGAGTGACCGGAACC	0.38
221	RM17754	5	TCACCCACCCTATCATTGTTTCC	CTAGCTGGTGACGACGATGAGC	0.44
222	RM17777	5	TAATTAGCAGAGCGTGACAAGG	CCAATCAATCGAGTTACTGTCTG	0.72
223	RM17799	5	TAATAGGAAGAGTGCGTCAGAGC	TCGACCAGTGATAACCAGTAACC	1.09
224	RM17823	5	ACATGGTCTCGCTAGATCTTTCC	CTTCCTCCATGATAAGCAAAGC	1.58
225	RM17855	5	CCTGTCCTCGTTGCTGGTAGC	CGCAAGAATGAAGGGAGAAGC	2.02
226	RM6517	5	GAGGATAGACCGGCCACAATCG	GCCTACGGCCCAGATGAATCC	2.85
227	RM17918	5	CCCTGCAACTATACTTGATCGATGG	GTTGTTGGAGCTTCTCGTCATCC	3
228	RM17923	5	CATGGCCAGTGCAAATTCAAGC	CACATTCTCTCCACTTTCTTGTCG	3.06
229	RM18119	5	GACGTTTAGCGGGTCAAAGAGG	GCTGGCATATCCCTAGGCTACC	7.18
230	RM18280	5	TAACACACTCGGCGGGTCAATCC	TTTGCGATAGAGGCCCGAAGACC	11.84
231	RM18359	5	ATGGCTCCCTGTAGCCGATGG	ATCGCCAACAACAACCTCTCAAACCTGG	14.01
232	RM18483	5	AGATTGCTTGTTTCGATGTGTGG	CGAAGTCAAAGACGAAGGTATGC	16.75
233	RM18768	5	CACATTTCCAGCGTTCTTGTTGG	CAGTTCTGATCCGTCTTTAAGCTTGG	21.37
234	RM18862	5	AGGGCACGTGCATTCCTAGAGC	GGAGAAGAAGGGCACATGCATCC	23.19
235	RM19001	5	GTTGCACTGCTGATCATGTACG	GGTTTACAAACCCTGAGGTTATCG	25.71

236	RM19156	5	AGAGGCCGGAATCCGAGAGG	CAAACCGTAGCCGAGGACATAAGG	28.62
237	JGT05-26.1	5	ATTCCTCTCGTTACGCCACGTG	TCCAGACCGGTTAACCCACCAG	26.1
238	RMES5-1	5	TATGATAGCGCCTTCGGAGT	GAGATTAACGTGCGCTCCTC	1
239	RM1169	6	AGCCAAGCAAGCAAAGCAAACG	GATTTGGGCGAGACGAGAACG	7.67
240	RM20068	6	GTGTTAGTGATGCATTTGTGG	GAGGGTCAAATAAGCAAACCTCC	16.31
241	RM20098	6	GGATACGCGATACGAGGATACG	TCAGCGTGAATTAGCTACAAGAGG	17.22
242	RM20378	6	ATGTCCTTCTTCGAGATTGCTTCG	AGAGCCCAACATATAGCGGATGC	23.94
243	RM20577	6	CTGTTGCTAGCTTACACGAATTGC	CCGGTAGTACGTACGCTACTATGC	27.5
244	RM3567	6	ACCACGCGTCATTGACATCC	ATGGGATGAAACTGCCACAACC	25.61
245	RM19367	6	CGTCATGTCGCGGAGGTAAGC	AGGCGTACGTGGAGCAGAGTGC	2.33
246	RM276	6	GTCCTCCATCGAGCAGTATCAGC	CTAGCAAGACATGGACCTCAACG	6.24
247	RM162	6	TTGTTCCAGTTCAGGTCTTGTGC	CCCTACAAACACCATAAGAAGCAACC	23.65
248	RM19660	6	TTTGTCCCTGCCGTACTTGC	AGCCACGTGGGTGAAATTAGC	6.75
249	RM19923	6	AGTGCCATACTCACATGACAAACC	GCCTTCGCAAAGAGGTATAAATGG	12.25
250	RM19569	6	TATAAGGGTTGGGAACCTTGTC	CAAGCCCATTATGAGTTAGAGG	5.4
251	RM20125	6	TTCAATACGGTACTTGGGCTTCG	CATCCACGTACTCCCTGTTACGC	18.41
252	RM20196	6	GTGGACCCACTACACAACATGG	TGTTCTACACTACGCCATTAGGC	20.06
253	RM19638	6	CCACACTGTACCGGTCGAAGACG	CTCTACAACTTGACGCCCTGTCAGC	6.38
254	RM20132	6	ACGCCGCTCTGCTCAAAGAACC	GTCGTGACACGGAGGCTTGTGG	18.76
255	RM20368	6	CAAGAATCAAGAGCCGAGAGTCC	TCTTCTGTACGGTTCTTGGTTGC	23.85
256	RM400	6	TTACACCAGGCTACCCAAACTCG	TTGCTGAGTTCCTCGTCTATCC	28.04
257	RM20536	6	ATCGTACGTGGAACCTAGACAACG	ACGACGACGATGTCCATTCTTACC	26.78
258	RM20623	6	GCTCGTACGGTGGGTGAATCC	GATGCGTCTTCTTAGGTTGAAAGC	28.14
259	RM19642	6	TCTCCGAGCACTTAAGAAAGTTGG	GAAGCGGTGATAGTGACACAAGG	6.43
260	RM6003	6	GGGTTGGTCACTCACAGCTTGC	TTCCATCACGTCTCTCGTCTTCC	2.1
261	RM19690	6	CCCACTGTCAATCCATTATGAAGC	ACATAAACAGGCATGTCTGAACG	7.47
262	RM19346	6	GATTTAGCTGGAATGCACCTTTGTGG	TGCGACGTGATGTCACGAATTATAGC	1.9
263	RM19381	6	AACGGGAGATCACAGGAATTTGC	GTGTTGCACTCGTCTCCATTTCC	2.5
264	RM20771	6	CAAACCTGCGTCTCTGTCTCTCTCC	GACGAGCACGACCCATCACC	30.66
265	RM3330	6	CGTTCGAGCAGAACCATCTACC	CCTCTTCCGCTCCACTCTCC	10.9
266	RM176	6	TACGACGTCTCCAGCCTCATCC	CGCTAATCAATCAGAGCGATGC	29.75
267	RM19391	6	GCTTTGTGTTACAGGATGTGCTGTCC	CAAAGCTTGGTACCTGCAAGACG	2.71
268	RM19665	6	CGATGTCTTCGAGTCCCTTAACAGG	ACGGTTGGTGATGCTCTTAGGC	6.92
269	RM190	6	GCTACAAATAGCCACCCACACC	CAACACAAGCAGAGAAGTGAAGC	1.76
270	RM162 (20355)	6	TTGTTCCAGTTCAGGTCTTGTGC	CCCTACAAACACCATAAGAAGCAACC	23.65
271	RM510	6	GTTTGACGCGATAAACCGACAGC	ATGAGGACGACGAGCAGATTCC	2.83
272	RM19426	6	CGGTGTCTTCTTTAAACAGC	ATGGATAAGCGGTATGTTCC	3.35
273	RM20060	6	CACACATGAGTGGTTAGGTAAGATGC	CAGTGACAAGAGCGAAATGATCC	16.06
274	RM20196	6	GTGGACCCACTACACAACATGG	TGTTCTACACTACGCCATTAGGC	20.06
275	RM1169	6	AGCCAAGCAAGCAAAGCAAACG	GATTTGGGCGAGACGAGAACG	7.67

276	RM19234	6	ACTCCCTCGTCTCTAGAGTTCTCC	GTTGGGAGTCTATCCCATCG	0.22
277	RM19292	6	TAGCTTGCGGTTAGGGAGACG	GGAGGAGATTACAGCTCTCCATCG	1.21
278	RM2434	6	ATTGAGGTGGACAAGAAGGGATGAGG	CGAGTTGGGAGGAGGTGATGAAAGG	4.32
279	RM19740	6	AGGTACATATTAACCCGAGTGC	AGTGTCCAAACATCTCATGC	8.43
280	RM19902	6	ACGAGTGCAGACACAATCACATGC	CACATCAAAGACTAATGCGGAAGACC	11.93
281	RM19980	6	AAAGGACAAGAGAGGTCAAGTGG	AAGGTTACCTAGAGGACCATAACC	13.53
282	RM20003	6	TGCACTATTGGCAGTAACATCG	GATGTGGATGGTATGAGAGTTGG	14.63
283	RM20023	6	CTGACCTGACGGCTGACATGACC	CAAGCAACCTTTCCGGGATTTGC	15.62
284	RM20096	6	CGGTAAGCCATAAATAGATCCCAAGG	TTTGAACAGCGACACGGTTTCC	17.21
285	RM20412	6	TGCCGGTAATTTAGTACGTATCC	CACTGTGGGAGTTCGCTTGG	24.68
286	RM20458	6	GTGTGGTGGTCTTGGGATGTTGG	TATAGCCCAGCAAGGGTGGTACG	25.45
287	RM20495	6	CATTCACACGCACATATCCTTCG	GAGAAGGGAAAGTGTGAAGAAGTTGG	26.16
288	RM20583	6	GAAGCTACTCCAAGTTCAGTCATTGC	TTAGTACGTATGCACATCCCATCC	27.56
289	RM20698	6	ACGGTCGTAGCAATAACTAGC	GGTCATAGGTCATAACTAGTCTGC	29.8
290	RM20773	6	TTGCCAATATTCCTCCAGTGC	GTTGTGTTGGGACCTTGATTCG	30.69
291	SSR6-6.9	6	TGGAGAGGAGGAGTGGATGCGTAC	TCTATTGGAGATGAACCAACC	6.9
292	RM1369(RM19321)	6	CATCGATTAGCTTACATGGCAACG	ACTAGTGCACCGTCTTCAATGG	1.56
293	RM19410	6	TGCTGATTGCTCACTACTTCATCC	GCGGGATACCATGGTCTAAAGG	2.91
294	RM586(RM19313)	6	TGCCATCTCATAAACCCACTAACC	CTGAGATACGCCAACGAGATACC	1.47
295	RM22031	7	GACTCACGGAACAGTATTAAGGTTGG	CACAATCCATGCACGTAAACTCG	26.33
296	RM21693	7	GCACAGACCAGAACTTTCTTCG	TGGCGAGTGTAGATGTAATTGG	19.45
297	RM21862	7	TTGTGGCCTTAATTGGGAGACG	CCGAGAAATTCGGTTAGTTGTTTCG	23.33
298	RM5720	7	GACTCGTCACTGACACTGATACG	CTTGTTAGGAAGAGCATTCTGC	28.61
299	RM5847	7	CTTTAGGTAGCGTCATCTTCC	TGGAAATACAGAAGGAGTCG	23.59
300	RM21789	7	CACCCATATATGCTTGTGGTGACG	AAGACGAATGGTCAAACAGTGC	21.79
301	RM21749	7	TAGCTTCCTACTTCCGCCTTCTATCG	ACAGTGGGAGGGAGTACATACACTGG	20.49
302	RM21521	7	TACTCGAGCAGGCAGAGCAACC	AGCATAGTCATCCAACCGAGACAGC	15.93
303	RM21260	7	CTGCACAACCAGGAGAAATTAAGC	CTGACCACTCTAGCTTGCCTACC	7.3
304	RM21103	7	ACAAGTCCACAAGGACCACAACC	TGCTCCACCCAAAGATACAGAGC	4.59
305	RM20784	7	GACCACATGCTTAGCTCCATCG	GCCAATATTGTGTCCCTTCAATCG	0.11
306	RM20800	7	ACAGGAGGGAAGACGGAGAGAGG	ATCACGGTCGCATACCGTTTCG	0.2
307	RM20813	7	CAAGTCCCAACAAGAAGAAGG	AGTCAGGAGAACCTACCTCACC	0.34
308	RM20827	7	GTAGTAGCATGTCCATTGTTGAGG	GCATTCTACTTCAGGTTTCTGC	0.52
309	RM20836	7	CATGGGTATGTTGGCTTCTTTCC	GCTGTTGCGTCCGAGATACTCC	0.6
310	RM20847	7	CTCGATCATCCGTCTTATATCTGG	GGGATTATTCTTGCCTTGATGG	0.76
311	RM20856	7	CAAAGATCATTGCGAGATTCC	TACACAGGTCAGGACACACTTGC	1.02
312	RM20873	7	GGGAGGTGCTCTCCCTTTCTCC	CCCTAGCAGTTGTTCTCTGGAGTAGC	1.25
313	RM20879	7	ACGTGCACCACCACCAACAGC	GGTCACCGAAGGGAAGAACAACC	1.29
314	RM20891	7	GTGGATGTACGTATAAGCATGTGTGC	GTACGGCTGACGGCTTTATTCC	1.55
315	RM20904	7	GATGGGTAGACTTGTATGATGAGC	GATTTGACCTGAGGTTTGACC	1.73

316	RM20926	7	CTCCCTCCCGAGATCTTGACG	GCAGGGTGCAGAAATAACTCTACAGG	2.02
317	RM20939	7	CCCTTTCAAAGTGGATATGCATGG	CAGCATTAGCACACAACAAACACACC	2.24
318	RM20953	7	TCCGGTAGCCTTCTTCTCACG	AAAGGTCAAACAACCCATGTCC	2.55
319	RM20958	7	TCTTGATCACACATTCACACC	AAAGTGGTTAGCCAACTTGTGG	2.55
320	RM20968	7	CTCCCACTTTGGGAAAGCAAAGG	AGGCTGCCTGATGAGGAGAAGG	2.74
321	RM20973	7	CGTGATGGTACAGGGAAGAAGG	CGCTACACAACACAACACAAATCG	2.8
322	RM21086	7	AGGCCCTTTATGTGGGTGTGC	CTTCTTCCTCTGCTTCCCTCTCG	4.18
323	RM21258	7	TATCATTCGGGTCCAAAGTGTCG	TCCGGTCCAAAGTCTCATTTGC	7.21
324	RM21437	7	TCGTGTGTTGTTCACTAGTGTGTCC	CTGTATGGATCAGAAGCAAGAAGC	13.31
325	RM21742	7	TGACTGATCCGTCTTGTGAGAGG	TTAAAGCCGGCACTGATGACC	20.37
326	RM21787	7	CCCTGTCTAAGTCTGTTTGACG	GCCGGTTTATATTCTTGACTGG	18.81
327	RM21808	7	TCCGATCGCTCCAGTATTCAGTGC	ACAGCTGCGCATGCAACAACG	22.13
328	RM21842	7	GAACGGGAGGAGGAGTTGTAGG	GACTTCATTTCAACTCGACGATGG	22.72
329	RM21222	7	GAGTTCGAACAGCATGATTGC	TAGCCCAATATATAGCGGATGC	6.61
330	RM22076	7	TGGCGACGATAGTGGAGGAGACG	CACCGGCAGCAGTCTCTGTTCG	27.52
331	RM22170	7	TTCTCCTTCCCTTCTTCCTTATGC	GTGGAGAAGAGGCAACCAAAGG	29.43
332	RM21596(RM6835)	7	GGTGGTAACCTTGAGAGCTACG	CGAAGAGGATAGATAACCCATAGTCC	17.63
333	RM1132 (RM21901)	7	TCAAGGTCGACATGTTAGGTATGC	AACCCTATCACCTGAGAAACATCC	23.93
334	RM22171	7	TAGTACCGCCATTACCATTTCATCC	GACGGTGGGACTCCTAATTACAGC	29.43
335	RM21539	7	GCCCAACTACTTCGACAGCTTCC	CAATGACCTGAGTAGCATCCAAGG	16.44
336	RM21435	7	ATCAACCACCTTACCGTGACTTCC	TCTTGCTCCAGGTAATCTTCATCC	13.2
337	RM21349	7	AACACAATTACCGGTCCTTAGC	GATCGATCTTTAGTCCCGGTTGG	9.9
338	RM21327	7	GTGGGAATTTGCTTGAAGTACC	CTGCTATTGTACCTGCTCTTATGC	8.89
339	RM21565	7	TATAGGTGGTAGCAACGCTGACG	GGTACCTCTCAAGCCAACAAGTATCC	17.04
340	RM21390	7	GGTAGGAGAAGACTCCGCTGACG	CACCTCGAAGGTGAGAATAATGATCG	11.01
341	RM21367	7	GACATGCACCAAGATGGTTCTGC	AAGGCTTCCTCGGCAGAAAGG	10.3
342	RM21770	7	GGATCGCAGATGCCTATATCTCG	TTCATCGTCTACAAGCCATCACC	21.17
343	RM22084	7	GGGCGGAGCTACATGATTCC	TGGTAGGAGGCATACAAGAGG	27.71
344	RM21039	7	TTCACTGTGGAAGTTCATTGG	AGACGATCGGACTCCTTACTGC	3.65
345	RM6697	7	TATCCCCGGGAGATCCAACAGC	AAGATCCAGTCGATTTGGTTCAGG	1.18
346	HRM23146	8	TGGTATCACCATTACCAACACC	TCCTTACGCTACCACGACTTCC	20.66
347	RM23612	8	CGATCGACCACAAGTAGAGTACG	CAATCACGTACAGGCAAGATCG	28.06
348	RM6699	8	TGGTATCACCATTACCAACACC	TCCTTACGCTACCACGACTTCC	20.66
349	RM1384	8	TGGTACGGGAGAACTGGTACGC	AATCGAGCCAGCCTAGCAAGC	11.84
350	RM22622	8	TAGGCCGTTCTGACGTAATACCC	CAGTGATGGTGATGCGATTTAGC	7.08
351	RM5933	8	AGCGATTGAGAACGAATCAACG	TGCCAAAGCTACACAAATCTGACC	27.46
352	RM531	8	GTTCCCACTCATAGTAAACCGATACG	CACGTTTCCTTCTTCAGATCATGG	22.46
353	RM544	8	GCTGCACCTCTCTCAATAAATGC	GTGGACAGCTCGAAACGAAGC	5.1
354	RM22549	8	GCCGTCCGATCTGCCATTAGG	TAGGGTTGGTGGCGTGCTTGG	5.41

355	RM3155	8	TGCGTGTAAGTGTTCGCTTGC	CCCTTGAGCTGATGCACATACG	27.89
356	HRM22892	8	GAACATGTCTTGGGTGTGATACAGG	TATGTTTAAACGGGCTCCAACC	14.65
357	HRM22622	8	TAGGCCGTTCTGACGTAATACCC	CAGTGATGGTGATGCGATTAGC	7.08
358	HRM22585	8	CACCGATTATTGTCGTATGG	AGTGAGGAAGGGAAGAATACG	6.08
359	HRM22977	8	ATGCATGGGCTAAATGATGAGC	TGCAGACCACAAAGAAGTTTGC	16.93
360	HRM23237	8	TAAAGCATTTGGACGGTGGATGG	GAGGTGGGTGTGACCCTTGG	22.32
361	HRM22299	8	ACGCTTCACATTGTAACACACAGG	GATCGATTGATCGGTGCTTTCC	1.2
362	HRM22732	8	TCTTTGAAGGTCATTCTCGGAACC	CGCCCTTAGCTGTGTTATTGTAATCG	9.88
363	HRM22839	8	ACGGTACGGATTATAGCGATGC	ACGCTCATCAACCGAGAAAGG	12.4
364	RM22866	8	TGCTTGTTCTTCCTCCCAAGC	CTTGCCCGTATTGTGGTCTCG	13.75
365	RM22837	8	ACCTGGGTCAGATGTCTGTTTGG	GGTAGAGCTCCATCCATCTTAGTGC	12.36
366	HRM23237	8	TAAAGCATTTGGACGGTGGATGG	GAGGTGGGTGTGACCCTTGG	22.32
367	RM408	8	AATTGCCCAACGAGCTAACTTCC	TGAGCTGTTTGTGCTCTTCTACTTCG	0.11
368	RM152	8	AAGGAGAAGTTCTTCGCCCAGTGC	GCCCATTAGTGACTGCTCCTAGTCG	0.67
369	RM23096	8	GTGCAATCATGTTACATCAGC	AAATAGACTACTGGGTGCGTTCG	19.92
370	RM22709	8	CGCGTGGGCGAGACTAATCG	CCTTGACTCCGAGGATTCATTGTCC	9.2
371	RM22585	8	CACCGATTATTGTCGTATGG	AGTGAGGAAGGGAAGAATACG	6.08
372	RM22772	8	GCCCGAAGAAATCAGAGTAGTAAGG	TTAGCAATATGGTGCCCAAGTCC	10.45
373	RM23237	8	TAAAGCATTTGGACGGTGGATGG	GAGGTGGGTGTGACCCTTGG	22.32
374	RM23518	8	TTTCGCTTCTGAGTTGGCAGAGG	GACCTGAACAACGTGCCAAACG	26.81
375	RM23605	8	TTCTCTAGATAGAAGTCTAGGCTCTGC	TGGATTAGCCGACTAGGTTAGAGC	28.01
376	RM22864	8	CGGGTGGTGTGAACCTATTCC	CACAACACAAGTCAACACAGTGC	13.57
377	RM23205	8	CCTGGGTATGGTAATCTTCG	ACAAGGCTATATGACCATGAGG	21.64
378	RM8005	8	CATTCCCAGCGTGGATATTCCG	CTTTGTCCATCTCGCCGTACTCC	
379	SSR8-19	8	GTGCAATCATGTTACATCAGC	AAATAGACTACTGGGTGCGTTCG	19
380	RM22225	8	TCCACCTGCTCAACTCCAAGAGG	GCACAAGAACTTGCCCGACACC	0.31
381	RM22231	8	TCTATCTCTTCCTCAGCTCCATGC	TATTGATCCTTGACAGGCTTTGC	0.42
382	RM22244	8	TACGTTTCGGTGGTCAATTGC	GCAGTACCAATCATTACCTTAGC	0.6
383	RM6863	8	CTACTGCCTCATTGCTCACATGC	TCAGGCAAGAACAGTTGATGAGC	2
384	RM22397	8	ATGGAGGATTCGTCGGGATTGG	CCGTCACTGTCTCCTCTTCATCC	3.07
385	RM22616	8	GACAAAGGGTGGAGAATCAATGC	GTCGGGATTAAATGGAAGGTATGC	6.75
386	RM22761	8	GAGGAGTTCGGCCGTATACTAGAGC	GCCACATTAACGCTATGTAGGATGC	10.29
387	JGT08-16.7	8	GAAAGAATTTGTTCCACGACCTC	GCTACAATTGCATGCTGGATGTCG	16.7
388	JGT08-10.2	8	GTAATAAACAGATGGAGCAGTACG	GTGGTACTGCTAATTAGGGGGTA	10.2
389	SSR8-2	8	CTGGGAACCAGGACGAAACC	ACACCTAAATTCCCGTGACTTGC	2
390	SSR8-10	8	GAGCTAATGTTTCTAGGCATGG	CACACAGATCCTAACACAGTCC	10
391	RM22554	8	TTGTCAAGATCATCCTCGTAGC	GTCATTCTGCAACCTGAGATCC	5.58
392	SSR8-18	8	GGTGAATTCTACTACGACGGATCG	GGTCCCGCTCATGACAATTAAGG	18
393	RM22362	8	GCCGATCGAGATTCTACTGATAAGG	GATAAAGAGCACCAGAGCGTTCC	2.66
394	RM23140	8	GGGAGCAGTCACCTAACATGTGG	GTGGCGCGTTAGGTTTCAGG	20.55

395	RM23959	9	TGCCAAAGCTAGCTACACACTCC	TGCATCATCTTCATCTCTCCATCC	7.9
396	HRM2454 (SSR9 18.1)	9	ATCCACAAGAGCACCGATGAGG	TGACCTGGTAGTGGTGAGTGTGC	18.11
397	RM24780	9	GA CTAGCCAGCCAAGGTTTGAGC	TGCTGCATGTGTGTATGTGACTACG	21.77
398	RM23901	9	TCAGCTATTGAGACAACGCAAACACC	TCGTAGGCAGGTGGCTATGACG	6.9
399	RM5526	9	CACATGATCCTCCACCCACTAGC	GCCTGGCCTCTCTTATCTGTCTACC	7.25
400	RM3917 (RM2848)	9	AACGAACGTGAACAAGCTAGGG	CCTGCTCCAATTCCTTCTATGG	15.59
401	RM107	9	TCTTACTGCGTCCTCTGGGTTC	ATTCTTGCGGCGATTTCATCTTCC	19.77
402	HRM24217 (SSR-9-13)	9	CAGAATCCAATAGGCTCCACACG	GTCAACGGCCACTTCAAGCTACC	13.04
403	HRM24654 (SSR9- 19.9)	9	TTGCTAGGTTAGCATCCGGTACG	TGGTCTTTGCGAATCTGAATCC	19.9
404	HRM24199	9	CTCGTAAGCCTAGGCCATCAAGC	AAACTTGAGCTTGGCTCGTTTCC	12.75
405	HRM23906	9	TTGACAAACTGGTAGCGTTGTGG	CCCAAGCCTCTAGTCATGATACTCC	7.03
406	HRM24481 (SSR9- 17.2)	9	GGCCCACGACTTAATAGACATCG	ACAGCGAACGTGGTGTCTCC	17.21
407	RM23766	9	AATCTGATGCCTGACCATTACGC	GAAACATTAAAGCCTCCCATGACG	3.58
408	RM24663	9	GTGATTGCCATTGATCAGCTTGG	GCAACCGATTCCCGTATAACAATACC	20.01
409	RM23819	9	CAATTCATAACGTCGGTCCTTCC	ATGGCTCTGTTTGCTGCATGG	5.01
410	RM23791	9	TCACCAACAAGTGGAGTACTTAGGC	CCTTACCTCAGGAGTGTTTCGATCC	4.26
411	RM23907	9	CACATATCGAGGGTGAAGAACC	AACCGGTGGACTCATTATTTCC	7.03
412	RM24094	9	GGAGTAATAGCAAGTTTGCACTGG	TCCACCAAATACATGTGGACTAGC	10.9
413	RM24199	9	CTCGTAAGCCTAGGCCATCAAGC	AAACTTGAGCTTGGCTCGTTTCC	12.75
414	RM24377 (SSR9- 15.7)	9	GGCTCCCATCGCCATTATTAGG	AGCTTAGCTTACAGCCGCATCG	15.75
415	RM23996	9	TACTTCTAACGCCGGCCAGTGC	ATCGTTTCTAGCGCGCAACACC	8.9
416	SSR-9-2.4	9	GGCGATACCTGCCATAGTTTCC	CCGAAAGCAATCTATGAGACACC	2.4
417	RMES9-2	9	ACGTTGATAAGCTCATTGC	GGGCACCGAAAATAAAATC	2
418	RM23683	9	GAGGGAGAGAGACGAAGAAGACG	CTAGCGATGGATTTGCGTTGC	0.91
419	RM23686	9	CCTAATGCGTGCCTCAAACC	GAGCTGCTCGCTACTAATAAGTCC	0.94
420	RM23724	9	CATGTGTCCAACCGTAAACATCG	AGTCGGTTTCGATTGACTTGTGC	2.13
421	RM23743	9	AGGTTTGCAGAAGCCAGAAAGAGC	GGCCGATGGTATGATGTGACC	2.67
422	RM23753	9	GGATTCTATTGGAGCCATACTCC	GTGCAGTGCAGATATCCAATACG	3.12
423	RM23785	9	TTCCTACGTGCTCACAAGGATAGG	AGCACGGGATATTAACAGGAAGC	4.11
424	RM23794	9	TCGGTGACTACATCAGGAACAGC	TGGATCAATCTTTCTCGCTTCC	4.33
425	RM23810	9	TATCAGAAAGTCCCAGTTCACC	TGTAGTACTGCAAGATGCAAGC	4.72
426	RM23824	9	AGCCTGACAGGTGTTCTATGG	CCTAGGAGCGTTTGTAGAGTGC	5.07
427	RM23835	9	TTCCGCTGTTTCTCTTCTGTGC	CTGGTTCTGCTGGTTCTGTAGTTGG	5.47
428	RM23855	9	TCTAGCATCTCGGAGACATTGC	ATGCACGGATGTTTGAAGAAGG	5.9
429	RM23899	9	GGAGGATCGAGGCGAAACTACC	AGCTGTTCGTGACCAGAATCTCC	6.88
430	JGT09-4.8	9	CGGGATACTAACAGCAAGCAAAT	TCTCGCACCCTAACAGGGAACA	4.8

431	JGT09-5.3	9	GCGTTTCTAGGGAGTTTAATGGA	GTTGGACATAGATGCTGGGTAA	5.3
432	SSR9-16.6	9	CGCAAGTGAATTCCATCTACTGAGG	AGGATGTCATGTCAGCGAAACC	16.6
433	SSR9-12.7	9	AAACTTGAGCTTGGCTCGTTTCC	CTCGTAAGCCTAGGCCATCAAGC	12.7
434	HRM24941	10	CGAATCTTGGAACACATCAACG	GGGAGGAGTGCTGTGAGAGG	1.91
435	RM25181	10	AAAGAGCTTCCCTAATGGCTTCG	GAGAGAATGACCTCTCCCAAGACC	8.44
436	RM25066	10	GTTGTTAGGTGTAGCCGTGTAGG	GTACACCAATAACTGTGGAAGAGC	4.25
437	RM25404	10	GCAACGGTTCTCCTTCCACTACC	CCATGATAGCGTTAGCCATAAACG	13.9
438	RM25661	10	TCTCTACCTGGCGTCCACTAGTTCG	AGGTGGACGTGCTCGATCTGC	18.48
439	RM4477 (RM25796)	10	GAAGCTTCCTCCTACGCTTTCC	TCAAGACTCAAGAGCCACAGTGC	20.65
440	RM5271	10	CGAATCTTGGAACACATCAACG	GGGAGGAGTGCTGTGAGAGG	1.91
441	RM25924	10	TTGCAGAGGACGATAAAATGC	CACCACACAAGAAACCTTAACC	22.49
442	HRM25453	10	GGCCATCAGTTTAAGTGTACG	ACTATGGGTCCTACCTATCTTCC	15.14
443	RM591	10	CTCATAGGTGGGTTAGTTTCTTGG	GCTGGTTTACAACCTGCTACTCTACC	22.45
444	RM25173	10	TAGTCACTGCCCTGGGTTAGAGC	GGAGCCCATACTCCGTAGAGTCC	7.93
445	RM25262	10	CAATGCAAAGTCTTGACGG	GCTACATTGCATAGATCACTCG	10.45
446	RM24944	10	GATCCGGGTTATCCTATACAGC	CTAGACTATGGTAGGTGTGATGTAGG	1.99
447	RM25143	10	ACCTTTGATCGAGGTAGGAGAGG	TACTAATTCCGTAGCGCCAAGC	6.88
448	RM25427	10	GGCGGTTCAAGAATAACTCATGG	TTACCAACCGGGACTAAAGATCG	14.6
449	RM25623	10	CCACATTAAGTGAAGAGACCATGC	TTGCCCTCATCTGTAATATGTCC	17.99
450	RM25661	10	TCTCTACCTGGCGTCCACTAGTTCG	AGGTGGACGTGCTCGATCTGC	18.48
451	RM25735	10	AGGCAGGCAAGCAGTAGTTTCG	ATCAAGATCAGGAGCCGCAAGG	19.56
452	RM24881	10	TGAGGGATAACCTGACCACACC	AAGGAGCACTGGTGAGAAACC	0.31
453	RM24898	10	CTATACATGCGTGGGAGAAGAGG	GACCATGTGGGATTCTATAAACG	0.69
454	RM24906	10	TGGCACCATTATGTGACACTGC	AGGATGCACCTCAACCTGTAACC	0.88
455	RM24916	10	AAGCTGTGAGGGTTCATTCCATCC	AGCAAAGTGGTAGGTGGGAGAGG	1.48
456	RM24922	10	GACACGCAGCACTGCTCACAAGC	GTGCGGGATGGGTTGGTTGG	1.55
457	RM24931	10	TCTCTCTCGTCCGAGTTGTTCCG	TGTACCTCTGTGCATCATCTCC	1.68
458	RM24945	10	GATCCGGGTTATCCTATACAGC	CTAGACTATGGTAGGTGTGATGTAGG	1.99
459	RM24965	10	CCACGTGTCATAAGCATCATCC	ATGGCGCACTTTCAATCTAACC	2.19
460	RM24969	10	GACCTGGTATATGTCCGTATCAGG	CTCGACAACGGGAAATCTATACG	2.32
461	RM24983	10	CTCCTCTCTCCGTCTCCTACTGC	GAGTAGAGTAGGGCTCACTCACAGG	2.53
462	RM24989	10	ACTATGAACGTGGCAGGAAAGG	CCTTGCTACTATAGACCTTCATCACC	2.75
463	RM24998	10	GTGAGGTGATGGATGAAGAAGATGG	GGATCCAGCATTGATGACAAGC	2.98
464	RM25023	10	GAGGGATTGGGCCCTAGGAAGG	GATGGACGATCACATGGCTGAGG	3.57
465	RM25054	10	CTTGCCACCACCACTGTTTCC	TTATCCATCCGCGGTATAAGTCC	4.1
466	RM25080	10	TGGAATAAACAAGGCGAAAGG	GACTAATGATGACTTCGAGGCTTACC	4.61
467	RM25092	10	CTATCTCCCTTGATGCGTACATGC	AAATCAGCGCGTGACAATTCCG	4.98
468	RM25104	10	TTCAACTCAGCTCCAGCAAGTACC	TACCGGGCATAATATAGACCTCAGC	5.58
469	RM25195	10	AAGTGCCATGTGGCTATTTAGG	GCAGGATCCACTGATAAATCC	8.81
470	RM25218	10	TGGCAGCCTCTATGTTAGACC	GATGCATATCGGTGATTTGG	9.27

471	ESSR-10-22.7	10	GCAGAGCGAGATGACCTACC	GCAAATTGACAGCAGATCCA	22.7
472	RMES10-1	10	CAGGGGGTACGTCTACAAGG	TATACCCGGCGAAATACGTC	1
473	10-12.6	10	CCAGGACACACTGTGAGTAGTTTGC	TTTCCCGTATGACAACAACACTGC	12.6
474	10-19.7	10	TTTCATTCTACCACCCAGCTTGC	GGACCTCTTCTTTCTCATGTTCTGC	19.7
475	JGT10-4.8	10	GTACGTACAAACCTGCCACCT	TGGACCCATCAGAAAACGACGCAA	4.8
476	RM24915	10	GCTGTGAGGGTTTCATTCCATCC	AGCAAAGTGGTAGGTGGGAGAGG	1.48
477	10-0.4.	10	CAAGAACCACCTTGGACGTATTGG	TCATATAGCCACGACTCCATCACC	0.4
478	JGT10-0.3	10	TGGCGATTTAGGAGCATTGTAGAA	GCCAACGACCACTCTCACCTAC	10.03
479	JGT10-4.3	10	TGGCGACTTAGGAGCGTTTGTAG	GCGGCACTTCCCCAAACAA	4.3
480	JGT10-5.7	10	TATGGAAGCAGCAAGTCGGAG	GTTGATCCTTCACGACCACCAA	5.7
481	10-21.2	10	ATGGACACGAACATGCAGAGAGG	AATGATTGCACGGTGTGATGG	21.2
482	HRM26213	11	GCCACAGGAGACAGCAAGAACC	CGATCCAATTCCAGCCTAGATAGC	4.72
483	HRM27183	11	GCAACCTGAACCCAAAGATAGC	GGTGGATTGAGATAGGCTCACC	24.93
484	HRM27322	11	AGAGCCCATGTAGCTACGCCTTCG	AATCATGCCGGCTGAAATTGTACC	27.55
485	RM5923	11	ATTCGGTCGATCGAGATAGTTGG	TGTCCGGTTTGCACAGTTGG	28.18
486	RM26550	11	GGGAAACATGGAAGAACACTCTAGC	CAAGCATTTCGAGCATTCAAGG	12.07
487	RM26558	11	GTTCAAATGAGGTCAGCCTATGG	AGAGTCAATGCATGCTCAAACC	12.6
488	RM27096	11	AGTTAGGATCGCTTCCAGGTTCC	TCCAACGGAATATCGTCTTGTAGGC	23.47
489	RM26784	11	CGTTATTGCGGATGACAGAAACG	GGCATGAGACACAACCAGATCG	17.53
490	RM26868	11	CAACTGTACTGTGCTGACCATCG	AGTAGGGACGAGGATTTTCATGG	19.15
491	RM181	11	TTTCAACTTGCTTCGCTTGC	ACCTCCTGCACTCCTTCATCC	2.02
492	HRM27323	11	GATCGATCTTTAGTCCCGTTGG	AACACAATTCACCGGTCTTAGC	27.56
493	HRM25970	11	TGTGAATCTGACTTGCCAATCG	AAGGCTGTTATGTGTCTTGCAACC	0.037
494	HRM26369	11	CGCATGCTCTCCAAATGATAAACG	CAACCGTACATGTAGTGGCACACG	8.16
495	RM26603	11	GATTCCGATAGAACGGAAGAGAGC	GAAGACCTCCTCACCAGTGAACC	13.74
496	RM26048	11	TCTTGGGTTCCGCATATCTAGG	CCCATCTCCCTTCTGTCTCTCC	1.94
497	RM287	11	GGCTACACCTACACGCGAGAACC	AGATGCATGGAATGCCTGTTTGG	16.61
498	RM536	11	TACCAGGATCATGTTTCTCTCC	ACTGTGAGATTGACTGACAGTGG	8.89
499	RM26616	11	GTGGAGGACGGTGATGGAGAAGC	ATTTCTTGACCGCCGCTGATTCC	14.09
500	RM27184	11	ATGTGACCTCGTCGATCTTGTTC	CCGAGTAGAGCAGCACACAGC	24.94
501	RM25970	11	TGTGAATCTGACTTGCCAATCG	AAGGCTGTTATGTGTCTTGCAACC	0.37
502	RM25982	11	GTCATCTGTCTGTCGGAGAACG	GCAGCATACATAAGCACTGACTGG	0.64
503	RM26352	11	TCACTGGATCTAACCAAGCCTACC	CAATCGCACAGACAAACACAGC	7.84
504	RM26829	11	GAGAAGGCCTGATGAGTACAAGG	GATTATTGTGCAGGTGAGAAGTGG	18.58
505	RM26972	11	ATTAACAGGGCGGAGCTAGAACG	CCCACAGCCATTGTCTCTACCC	21.16
506	RM27317	11	AGCGAAGCGCATGGTAATGACG	CTGCCAGCATGACCGAAATCTTCC	27.49
507	RM26048	11	TCTTGGGTTCCGCATATCTAGG	CCCATCTCCCTTCTGTCTCTCC	1.94
508	RM27369	11	ACATATCGACGGTGGATGAGAGC	TCCGTGTGCATACATTCTTGAGC	28.18
509	RM26784	11	CGTTATTGCGGATGACAGAAACG	GGCATGAGACACAACCAGATCG	17.53
510	JGT-11-8.9	11	TGGAACACCCCATAGACAAACAGC	TGGCAAGTGGTATTCTTCCTTCC	8.9

511	JGT-11.5.8	11	AAGACAAGGAGGTTCCAGTGTCC	GCCCTAAACCCAAATAGAAGAACG	5.8
512	JGT-11-15.3	11	GATCGCCCGTTAGCCTTTTCGA	GAACAGTTTTCGTGGCAACAA	15.3
513	JGT-11-11.4	11	AGAGCATCTGTGTTGGTAAGTTGG	CGAGGCATCTTTGAGGTCTATCC	11.4
514	RM26889	11	CATGTAACCTCCTCTTGCCTCTGC	ACGTACTTAGCTTGTGCGATCC	19.55
515	RM26901	11	GGTACAGATCGACTGACTATTACTCC	GCATGCATATACGGTACTTTCC	19.72
516	RM26907	11	AGGAGTAGCTGTGGCCTGTTCC	CCTGCGAGCTGAGATGAATCC	19.87
517	RM26932	11	TTGTTTAGCTAGACTGCACTGG	TACAGTGTGGTTGGAAC TAGAGC	20.48
518	RM26951	11	GGAGCGAGAGAGGGAGAGAAGG	GATAGATTAGGGACGCGACTACATCG	20.72
519	RM26969	11	CTCACACTTGCAACATCCTAGC	AAGGCTCTAGTTGGTGAAGACC	21.11
520	RM27022	11	TGGACTACGAATAATGGTGGTCAGC	CTAAGCCGGCACTGTGACAACG	22.08
521	RM27085	11	GATGAGGCTGGTGAGCATGACTGG	ATGTTGCCGGACTTGAGGTTGC	23.34
522	RM27201	11	GCCTACTGGTCCAAGGTGAAGC	CAAGTAGACGTCGTGGAACATGG	25.09
523	RMES11-1	11	TTGACCTAGCCAACCTCACC	CGGAGTACACGTCCACCAC	1
524	JGT 11-0.9	11	CGATTGATCCCGTGCAAGTAGG	CATGCTAGTGCATTCTGCGTAGG	0.9
525	JGT 11-9.6	11	GCTCCTGCATTTAGGCGACTCC	TTGTCTCAACCACCCAAGAAAGC	9.6
526	JGT 11-21.1	11	CTCACACTTGCAACATCCTAGC	AAGGCTCTAGTTGGTGAAGACC	21.1
527	JGT 11-20.5	11	AGAGGATGAACTACAGGGCAAGC	GTGAGGTGGGAAC TAATCCATCG	20.5
528	JGT 11-4.2	11	TGGTGAGAGGGCTAGAGAGATCG	AATGTAAGGGTGATTGCCGACATGG	4.2
529	JGT 11-1.5	11	AAGAGGACCTCGAGGATGTACCG	CGTCAGCCTGTCTTGTGTACTCG	1.5
530	JGT11-19.9	11	TCTGGAATGTGTCTTGGGGGA	CTAGGTTTAGGTGACCAACAGTC	19.9
531	JGT11-16.3	11	GGCGGCGTATTAGCGTTGTA	AGGTCTAGCCCATGTTAAATCTTCT	16.3
532	RM5479	12	CTCACCATAGCAATCTCCTGTGC	ACTTCGTTCACTTGCATCATGG	24.44
533	RM28039	12	CGAAAGGGCATATAGCCTAGTCC	ATCTTACCATTGCGCTTTCTCC	13.79
534	RM28067	12	GAGGGAGTACTAAACTTCCTAACG	ATGTGGATGTGATCTCCATAGG	14.74
535	RM28157	12	GCTTAATTCTGACAGACCAGTGC	GATCTAAACACAGCCTTCCTTGG	17.43
536	RM28559	12	TTGTGCGTACTTGCTTGTCTATGG	CTGTTGTTGTTTGCCGCTAATCC	24.05
537	RM27879	12	GCTGTAATTGTACGGCTCCAACC	ATAACGAGCTGATCGCAATGTGG	9.27
538	RM28424	12	TCCACACACTTCGCCAATAAACC	CCGCCACCACTCCTCTATCC	22.4
539	RM2935	12	CTTTGCACATGCGTCGTTGC	AAACTGCACATGGCCTCTCAGG	7.42
540	RM28698	12	GAGAAGCTCCGCACTCAGAGACG	CCAGCCCAGCTAGCAACTTAGAGC	25.88
541	RM2851	12	CAGTGCTTTAAGTGTCATCTCC	CAGCAAGAGTAGCGACTATATGTACC	0.75
542	RM27601	12	GCTTAGATGGTGACAGGAAACC	GCTGTGTTTGTGTTGAAGGTGTTGG	3.55
543	RM1047	12	CACCTGGCTCCTCATCAAGTACC	TGGCTTAGTCTTGTGGAGACACG	11.22
544	RM28346	12	GCCCAAAGTTAATATCGGTGTCTCC	AGCCTGCCTAGCACTCATAGACC	20.98
545	TRS-26	12	GGAGAGCCAATGTGATAAGGA	CAACAAGAGAGGCAAATTCTCA	26
546	SSR12-27.5	12	GACGTACAGCCTCCAAATGAAAGG	CTCCATCGTTCACCGAAAGTGC	27.5
547	RM27457	12	CAATTGGCAGTGCAGTGGTAAGG	GCTCTGTTCACGAGTCTGTCTCTTCC	0.98
548	RM27471	12	CAAACGGACACGCAAGAGAGC	TTGCTTGGGTCTAAACGTGAAGG	1.35

549	RM7582	12	TCTGGAAGGGAAACAACGTACC	AGTTGAAGAAGCTGTAGCCAGATCG	1.5
550	RM27491	12	CTGGACGCCACTCTGCTTCG	CCCACAGCATGGTGTAAGACTTGC	1.62
551	RM5927	12	TGTATAGCCCGGAAGTATGATCC	TCTGGTCTCGTCTCTCATGTGC	2.2
552	RM27547	12	CCTTGAAGAGGAAGAAGGTTTGC	CCTTGCTCCTCATATAACGACAGC	2.95
553	RM27458	12	GCTCGCCTGCCTTTAATACTCC	GAGAAGTAAACACGCCACCAACC	1.03
554	RM6690	12	AGGGAGTACCTTGTAACGAATTGC	GCATCAATTACACCAGTGAGTAGC	1.44
555	RM6296	12	TTAAGCCCACGTTTCTCTTGTC	CTCGCTAGGGTTAGGGTTTCAGG	3.2
556	RM27577	12	AGTGGAAGCCCAAGCCAGAGG	CGTGACGTCTCCTTCTTTCTCACC	3.3
557	RM27580	12	TGATCGCCAGGATCTTGCTCTCC	GGGCTCTGCCTTATCGGATCG	3.32
558	RM27596	12	ATCCCGACCGTCCAGTTCAGC	GGTCTCGATCTCTTCGATGACTTGC	3.5
559	RM27609	12	TGTTCACTGGTGGTTCTGATTCC	CCACCAAAGATTAAGGGTGACTATGG	3.62
560	RM27658	12	ATCATGGGACAGCACAACAGTGG	TTCAATTAGGCCACGCCTACTAACC	4.41
561	RM6998	12	GATAAGCTTGC GGACCGGATTAAGC	CTCCACGTTTGGGTCGCTACTCG	4.74
562	RM27699	12	TGATGGACGTTTAAGTCGGATAGG	GAGGTCCAGGAAATTGATAGATGG	4.96
563	RM27730	12	CCAGTTAATTGGGTTGGTTCATGG	CGGGACGAATAATAGATGGATGG	5.59
564	RM27822	12	GTCGTCGAGGTCGAGGGTTAGC	ACAGGAGCACGACCAGAGAAGC	7.64
565	RM27841	12	TAAATACCCGACAATGCCCTAGC	GGAAATCCCATCAATCACAAGAGC	8.02
566	RM27862	12	TGCTAGCATGCTCATCCACTTGC	CAAGGGAACACCGTGTATTGTTTGC	8.65
567	RM27871	12	AGGCTAGGATGAGCAGGAGAAGG	GGTGGGCCCTATATTCCAAGC	8.83
568	RM28275	12	ATTCCAACCCAACCTCCAAATTCC	GTTGGGCTGTACTGTACGTCTTGACC	19.47
569	RM519	12	AATTTCCGCGAAATCAGCATCC	TCATCTGGACAGTCGAGGTACGC	19.97
570	RM27406	12	TGGTAGGTGTGCAATAGAAGTAGG	AATGCATGCAAACACAGTGG	0.22
571	RM27876	12	TGCAAATAGTGCTTCACTGACC	CAGCATGCATAGAGAAGAGTTGG	9.11
572	RM27959	12	TGGAGATGGGTGGACGGTTAGG	ATTTAGCGAAGCGGTTGGTAATGACG	12.01
573	RM27966	12	TCTGAGCCAACAGTAAGAGTCAGG	TGTCACCCGTAGTGTGTGTACGG	12.16
574	RM28011	12	CGCTATAATAGGCCGCTATTGG	GCACTATTGCGAATGCTAAGTTCC	13.55
575	RM28110	12	GATCGATCTTTAGTCCCGTTGG	AACACAATTCACCGGTCCTTAGC	16.3
576	RM28277	12	TGCACCACCTATTTCAATCCACTCC	CCTTCTCAAGGGAAATCACAGAAGC	19.49
577	RM6217	12	CGCAGATGGAGATTCTTGAAGG	ACAGCAGCAAGAGCAAGAAATCC	22.7
578	RM28461	12	GAGCTTAGAGGCTGTCCTTGTC	GCCTGAGTCACGTTTCTTATCAGC	22.93
579	RM28465	12	GCCTCGTAGAGACTCAAATAAAGC	GTAGCTAGCCACCAATAGCATGG	22.98
580	RM28469	12	GCTCACAGATATGAGTTCATGG	CCTCACAAATGTTAGTGGAAGC	23.03
581	RM28472	12	GCTATGAACCTGTACACATGTAGG	GAGCACCATAACAATCAGTTGC	23.06
582	RM28481	12	GTCAATTAACCATTGCCCATGC	TTCACGTGGGAATACTCATGC	23.17
583	RM28484	12	TACTGGAGCCACTGGTTATTAGC	CTTAATTTACCGCAGCAACG	23.17
584	RM28487	12	GAGGTGATCTTAATGCCATCTTGACG	TACATGCAACCTGGGTATGAGAGTGC	23.2
585	RM28616	12	CACCGGAGTTCCTCAACTTACC	TACGTATGGCCAATTCAGACTGG	24.91
586	RM27514	12	CCGGTTCGTAACCTCTTTAGTCC	CGACTACTACTCATCGAAATCGTAGC	2.32
587	RM28260	12	CGGAAACAATCGGAGTCACC	CTCTCGGCTTCTTCTCAATCC	19.18