

Role of *Oryza sativa* G2-like Transcription Factor Family in Low P Tolerance in Acidic Soil Adapted Rice Genotypes

Thesis

Submitted to the
Central Agricultural University, Imphal
in partial fulfillment of the requirements
for the award of the degree of

Doctor of Philosophy

in

Plant Molecular Biology and Biotechnology

by

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CAU/684-A/08(B)



**SCHOOL OF CROP IMPROVEMENT
COLLEGE OF POST GRADUATE STUDIES
IN AGRICULTURAL SCIENCES
CENTRAL AGRICULTURAL UNIVERSITY (IMPHAL)**

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March 2019



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Certified that **Mr. Karma Landup Bhutia** {Registration No. **CAU/684-A/08(B)**} has satisfactorily prosecuted his course of research for a period of not less than four semesters and that the thesis entitled **“Role of Oryza sativa G2-like Transcription Factor Family in Low P Tolerance in Acidic Soil Adapted Rice Genotypes”** submitted by him to the Central Agricultural University, Imphal - 795 004 (Manipur) in partial fulfillment of the requirements for the award of the degree of **Doctor of Philosophy** in the subject of **Plant Molecular Biology and Biotechnology** is the result of original research work conducted by him under my supervision and is sufficiently of high standard to warrant its presentation to the examination.

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I hereby declare that the thesis entitled “**ROLE OF ORYZA SATIVA G2-LIKE TRANSCRIPTION FACTOR FAMILY IN LOW P TOLERANCE IN ACIDIC SOIL ADAPTED RICE GENOTYPES**” is an authentic record of the work done by me and that no part thereof has been presented for the award of any other degree, diploma, associateships, fellowship or any other similar title.

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ACKNOWLEDGEMENT

First of all, it is the “The almighty” before whom I bow my head with great reverence because without his blessings this tedious and wearisome task could not be completed.

I am overwhelmed to avail this rare opportunity to evince my profound sense of gratitude and regards to Dr. Wricha Tyagi, Professor, School of Crop Improvement for her meticulous guidance to me. As a chairman of my advisory committee, it was her regular supervision, constructive criticism and calm endurance that helped me in completing this investigation and preparation of manuscript in this form.

Under the expert eyes of the members of my advisory committee, Dr. V.K. Khanna, Professor, Biotechnology, SCI, CPGS, Dr. Mayank Rai, Professor, Genetics and Plant Breeding, SCI, CPGS, Dr. Dipali Mazumdar, Associate Professor, Bacteriology, SCP, CPGS, Dr. Devyani Sen, Assistant Professor, Genetics and Plant Breeding, SCI, CPGS, my endeavor has been rendered into the present form.

My sincere thanks are due to Dr. N.B Singh, Dean, CPGS and Dr. V.K Chowdhury, Chairman, SCI, CPGS for providing me necessary facilities.

I would like to acknowledge the Ph.D scholarship (NFST) from Ministry of Tribal Affairs, UGC, GoI. I would also like to also acknowledge funding from projects sponsored by DBT, GoI (Unit of excellence) and ICAR, GoI (NAIP, component IV) for the materials generated and consumables procured under these projects helped me to complete my research.

I extent my thanks to Dr. Noren Singh, Professor, GPB, SCI, CPGS, Dr. Ng. Tombisana Meetei, Assistant Professor, Plant Tissue Culture, SCI, CPGS, Miss Soringla, Staff, SCI, CPGS, Tamo Imocha, Staff, SCI, CPGS, Kong Nadia, Staff, SCI, CPGS, Erneica, JRF, SCI, CPGS, Kong Mary and other field workers for their kind cooperation at the time of need.

I am thankful to my parents, and Agya Dadul, Yapchung, Bainila, Nangsol for their affection and guidance.

I also wish to record my warmest thanks to my friends, batchmates, senior and loving juniors and all the beloved and respected people who helped me directly or indirectly during the course of this experiment and for making my stay at CPGS, Umiam, a beautiful experience.

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Umiam

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LIST OF ABBREVIATIONS

Symbol	Full form
%	Percentage
°C	Degree Celsius
μL	Micro litre
C	Control
cm	Centimeter
DAT	Days after transplanting
EDTA	Ethyl diamine tetra acetic acid
PCR	Polymerase chain reaction
PUP	Phosphorous uptake
PUE	Phosphorous use efficiency
g	gram
HCl	Hydrochloric acid
HvSSR	Highly variable simple sequence repeats
LR	Lowland rice
P	Phosphorous
PR	Phosphorous response
QTL	Quantitative trait loci
rpm	Revolutions per minute
SDS	Sodium dodecyl sulphate
Ct	Critical difference
TE	Tris EDTA
TBE	Tris borate EDTA
Nm	Nano metre
μM	Micro molar
GLK	G2-like transcription family
OsGLK	Oryza sativa G2-like transcription family

Abstract

Rice productivity is affected by availability of phosphorus (P). Under acidic soil condition, phosphorus (P) is rapidly immobilized in the soil due to its high reactivity with cations. Therefore, to increase rice productivity, it is important to develop rice varieties tolerant to low P through identification and introgression of genes attributing to low P tolerance. The present study was conducted to characterize a transcription factor family *i.e.* G2-Like (*OsGLKs*) through *in silico* and wet lab approach using quantitative PCR (qPCR). *In silico* results showed that, MYB transcription factor family *OsGLK* belongs to super family GARP. The molecular function of the *OsGLKs* is sequence-specific DNA binding. On the basis of biological functions three categories *viz.*, a) two-component response regulator (*OsGLK4*, *K7*, *K10*, *K27* and *K42*), b) putative transcription factor (*OsGLK1*, *K2*, *K5*, *K8*, *K18*, *K19*, *K21*, *K29*, *K30*, *K33*, *K34*, *K35*, *K37*, *K38*, *K39*, *K44*, *K45*, *K50* and *K51*) and c) most importantly phosphate starvation response (*OsGLK6*, *K14*, *K15*, *K23*, *K31*, *K32*, *K36* and *K40*) could be identified. Seventeen members also carry multiple copies of P1BS (PHR1-binding sequence) *cis*-acting element within 1kb upstream from CDS sequence in 5' UTR region suggesting regulatory role in response to P levels. Ten genes are found to be differentially expressing under low P conditions. Gene *K4* is up-regulated in susceptible genotype (BAM 350), *K41* is down-regulated in BAM 350, *K8* is up-regulated both in tolerant genotype (LR 26) and susceptible genotype (BAM 811), *K7* is down-regulated in LR 26, *K19* is up-regulated in LR 23 and BAM 350, *K30* is down-regulated in tolerant genotype (LR 26) and up-regulated in susceptible genotype (BAM 350), *K15* is down-regulated in LR 23, *K14* is up-regulated in LR 23 and BAM 350 but down-regulated in BAM 811, *K37* is up-regulated in LR 23 and BAM 350 and *K10* is up-regulated in both the tolerant genotypes (LR 23, LR 26) and down-regulated in both the susceptible genotypes (BAM 350 and BAM 811). The present study also used marker trait association to identify alleles, genes and genotypes for better performance under low P acidic soils in a bi-parental mapping population (F_2) derived from two P deficiency tolerant genotypes; Sahbhagi Dhan (LR 23) and Chakhao Poreiton (LR 26). Selected genotyping was performed on 20% of the population (identified on the basis of 5 yield contributing traits) using different types of markers like candidate gene-based, SSR and SNP, including markers belonging to rice *GLK* family. The qPCR data and marker trait association has identified chromosome 2 to be of key importance in low P response. The chi square and regression analysis revealed markers associated with different yield attributing traits. HvSSR02-10 is associated with TN 60 ($R^2=0.0548$), FG ($R^2=0.0901$), F % ($R^2=0.1271$), GYPP ($R^2=0.1214$), PUE at harvest ($R^2=0.0823$) and PUE at 60 DAT ($R^2=0.1044$). Marker HvSSR02-14 is associated with TN 30 ($R^2=0.0806$), TN 60 ($R^2=0.0531$), FG ($R^2=0.1105$), F % ($R^2=0.1611$), GYPP ($R^2=0.1349$), PUE at harvest ($R^2=0.0891$) and PUE at 60 DAT ($R^2=0.0845$). HvSSR02-33 is associated with FG ($R^2=0.103$) and GYPP ($R^2=0.0797$). Markers HvSSR10-30, 10-34 and PR1-2 are associated with PN ($R^2=0.0657$, 0.0943 and 0.0604), respectively. Candidate gene based markers PR03102 and 3103 are associated with F% ($R^2=0.0564$). PR9-2 is associated with TN 30 ($R^2=0.0532$), F % ($R^2=0.0597$), GYPP ($R^2=0.0776$) and PUE at 60 DAT ($R^2=0.1004$) while PR096-4 is associated with PUE at 60 DAT ($R^2=0.0593$), snpOS305 is associated with FG ($R^2=0.0718$), F % ($R^2=0.122$), GYPP ($R^2=0.120$) and PUE at harvest ($R^2=0.0712$). Marker snpOS316 is associated with TN 30 ($R^2=0.0604$) and TN 60 ($R^2=0.053$). For all the markers, Sahbhagi Dhan type allele was positively associated with better performance except for the markers FR033-3 (field grains per plant) and PR1-2 (TN60), where the SD allele was negatively associated, and for marker 07-44, CP was positively associated with spikelet fertility %. The qPCR data and marker trait association has identified a nearly 1.63 Mb regulon on chromosome 2 to be of key importance in low P response. The genes and markers identified on this chromosome will be key to imparting tolerance to low P in diverse genetic backgrounds under acidic soil conditions.

Key words: GLK, rice, low P, acidity, yield, bi-parental population, molecular markers

Chapter 1

Introduction

Rice (*Oryza* species) is a self-pollinated monocot plant belonging to Poaceae family.

Area :	world- 161.63 Million hectares (2016-17)
	India- 43.99 Million hectares (2016-17)
Production:	world- 487.37 Million metric tons (2016-17)
	India- 109.60 Million
Productivity:	World- 4.50 Metric tons per hectare (2016-17)
	India- 3.74 Metric tons per hectare (2016-17)

(Source: US Dept. of Agriculture (USDA, 2018))

It is the world's most important food crop and primary source of food for major portion of the world's population. According to Maclean *et al.* (2002), source of more than two thirds of the calorie intake among the poorer populations of tropical and subtropical countries is rice. So, in order to feed the ever growing world's population, higher production of rice is must. But the productivity of rice is adversely affected by many biotic and abiotic factors.

Focusing on abiotic factors, nutrient deficiency and mineral toxicities are the major problems affecting rice productivity. Both these problems are mainly associated with soil pH especially acidity. According to Brunner and Sperisen (2013), about 40 % of the global aerable land is acidic. And in south and south-east Asia, more than 100 million ha of land is unused which is suitable for rice cultivation due to soil acidity and problems associated with soil acidity (Ismail *et al.*, 2007).

In India alone, 49 million ha of the total land area is affected by soil acidity out of which 24 million ha have pH below 5.5 (Kumar *et al.*, 2012). And major portion of acidity affected soil are concentrated in north-eastern part of India due to various reasons most importantly heavy rainfall with about 65 % of its area under extreme levels of soil acidity (pH below 5.5). Productivity potential of acidic soils is estimated to range from 25-80 % less than normal soil (Singh *et al.*, 2011). Toxic and deficiency effects (aluminium and iron toxicity, phosphorus (P) deficiency) of acid soil

results from an interaction between pH and elements in the soil, leading to poor growth and yield of rice.

1.1 Phosphorus deficiency

Phosphorus is the second most important macronutrient after nitrogen required for plant growth and development. Under acidic soil conditions, phosphorus becomes unavailable to the crop plants as it gets coupled up with metals like aluminium and iron. According to Lopez-Arredondo *et al.* (2014), approximately 70 % of the world's total cultivated land, including acidic and alkaline calcareous soils is affected by phosphorus deficiency.

Under acidic soil condition, phosphorus (P) is rapidly immobilized in the soil due to its high reactivity with cations like aluminium and iron, thereby becoming unavailable to the crop plants. Deficiency of P causes adverse effect on crop productivity including rice. P deficiency leads to stunted growth of rice plants with reduced tillering, narrow, short, very erect and dark green older leaves and reduced number of leaves, panicles and grains per panicle (Vinod and Heuer, 2012) and ultimately leading to poor grain yield. P deficiency may also cause delay in maturity, high sterility and poor grain quality.

To increase the rice productivity under low P conditions farmers can use phosphatic fertilizer as a source of phosphorus for growing rice plants but the limitations are that it is a non-renewable natural resource and also regions of low phosphorus are mostly found in the developing countries and procurement of phosphatic fertilizers becomes a costly affair for the farmers.

Another strategy to increase rice productivity is by developing rice varieties tolerant to low P conditions through identification and introgression of genes attributing to low P tolerance. Tolerant plants exposed to P deficiency activates a range of mechanisms that either result in increased acquisition of P from the soil or a more efficient use of the internal P (Vance *et al.*, 2003).

Numerous studies have been done to identify the genes/loci responsible for P deficiency tolerance. However, the molecular mechanisms that regulate gene expression during P starvation remain poorly understood; with only few transcription factors identified that each influence only a part of the spectrum of changes induced by P deficiency (Rouached *et al.*, 2010). The present study was conducted to characterize a transcription factor family (G2 like or *GLK*) using *in silico* and wet lab approach to look into its role in P deficiency tolerance.

1.2 *Oryza sativa* G2 like transcription factor

G2 stands for golden 2 gene family which was first identified after the isolation of a pale green maize mutant that exhibited perturbed development of photosynthetic cell types. G2 gene regulates the plastid biogenesis in all photosynthetic cells and plays a critical role in the development of chloroplasts in C3 mesophyll and C4 bundle sheath cells (Langdale and Kidner, 1994). Sequence analysis suggested that G2 acts as a transcription factor (Hall *et al.*, 1998). Similar to G2 genes, G2-like transcription factors (G2-like TFs) are involved in chloroplast development and have been reported to co-regulate and adjust the expression of a set of nuclear photosynthetic genes and thus act to optimize photosynthetic capacity in different developmental and environmental conditions (Waters *et al.*, 2009). As the low P levels change the leaf structure and colour, we wanted to explore the possibility of GLK members having a role in imparting tolerance under such adverse conditions.

Present study also aimed to use marker trait association to identify alleles, genes and genotypes for better performance under low P acidic soils in a bi-parental mapping population (F₆) derived from two P deficiency tolerant genotypes (Sahbhagi Dhan (SD) and Chakhao Poreiton (CP)) with the following objectives;

- To characterize *Oryza sativa* G2-like family using *in silico* approach.
- To characterize *Oryza sativa* G2-like family with respect to low P response in Sahbhagi Dhan and Chakhao Poreiton.
- To associate candidate genes including *Oryza sativa* G2-like family with low P tolerance in RIL derived from Sahbhagi Dhan and Chakhao Poreiton

Chapter 2

Review of Literature

2.1 Introduction to rice

Rice (*Oryza* species) is a self-pollinated monocot plants belonging to Poaceae family. It is the world's most important food crop and primary source of food for major portion of the world's population (Huang *et al.*, 2010). It is source of more than two thirds of the calorie intake among the poorer populations of tropical and subtropical countries (Maclean *et al.*, 2002), 21 % of the calorie intake among the world's population, and more than 80 % calorie intake in some South-East Asian countries (Wu *et al.*, 2011). However, the production and productivity of rice is not increasing at the pace of ever growing population and their demands for food (Ali *et al.*, 2018), with the average production of 487.37 Million metric tons and productivity of 4.50 metric tons per hectare in the world and production of 109.60 million metric tons and productivity of 3.74 metric tons per hectare in India (2016-17) (USDA, 2018).

The production and productivity of rice is adversely affected by many biotic and abiotic factors. Focusing on abiotic factors, nutrient deficiency and mineral toxicities are the major problems affecting rice productivity. Both these problems are mainly associated with soil pH especially acidity (Das *et al.*, 2017)

2.2 Soil acidity

About 40 % of the global aerable land is acidic Brunner and Sperisen (2013). In south and south-east Asia, more than 100 million ha of land is unused which is suitable for rice cultivation due to soil acidity and problems associated with soil acidity (Ismail *et al.*, 2007). In India alone, 49 million ha of the total land area is affected by soil acidity out of which 24 million ha have pH below 5.5 (Kumar *et al.*, 2012). And major portion of acidity affected soil are concentrated in north-eastern part of India due to various reasons most importantly heavy rainfall with about 65 % of its area under extreme levels of soil acidity (pH below 5.5). Productivity potential of acidic soils is estimated to range from 25-80 % less than normal soil (Singh *et al.*, 2011). Toxic and deficiency effects (aluminium and iron toxicity, phosphorus (P) deficiency) of acid soil results from an interaction between pH and elements in the soil, leading to poor growth and yield of rice. One of the major problems associated with soil acidity is conversion of phosphorus into compounds which becomes unavailable to crop plants.

2.3 Phosphorus deficiency

Study on agronomic phosphorus imbalances across the world's croplands suggested that in total, 29 % of the global cultivated area had overall P deficits and 71 % of the cultivated area had overall P surpluses (Fig 2.1) (MacDonald *et al.*, 2011). Although, the regions like parts of south eastern Asia, eastern and northeastern part of India comes under moderately P surplus region; the phosphorus in the soil becomes unavailable to the crop plants because of the acidic nature of the soil where P is fixed by metal oxides (Kochian, 2012; Zeng *et al.*, 2016, Das *et al.*, 2017). The deficiency of P leads to drastic change in plant metabolism resulting into poor growth and development. Deficiency symptoms of phosphorus include stunted growth, reduced leaf size, reduction in number of leaves premature senescence of leaves and delayed flower initiation (Angus *et al.*, 1990; Bolan *et al.*, 1985 and Marschner, 1996).

2.4 Role of phosphorus in rice plant system and its uptake from the soil

As like in other plants, phosphorus in rice plants is involved in structural (phospholipids), storage and transfer of high energy compounds, the most common compound being ATP (adenosine triphosphate); a constituent of a number of structural units of plants such as DNA, RNA and phosphoproteins, and also act as a regulatory role in plant metabolism and influences the activity of enzymes (Marschner, 1996).

The form of P most readily accessed by plants is phosphate (Pi), and the concentration of Pi rarely exceeds 10 μM in soil solutions but plant requirements are high (Bielecki, 1973). Phosphorus is taken up by plants as HPO_4^{2-} and H_2PO_4^- (Marschner, 1995). Phosphorus moves from the root to xylem and then transports to the above ground organs symplastically. Under high Pi condition, plant store Pi in the vacuole and from there it is remobilized to other cellular compartments to maintain cellular homeostasis (Pratt *et al.*, 2009).

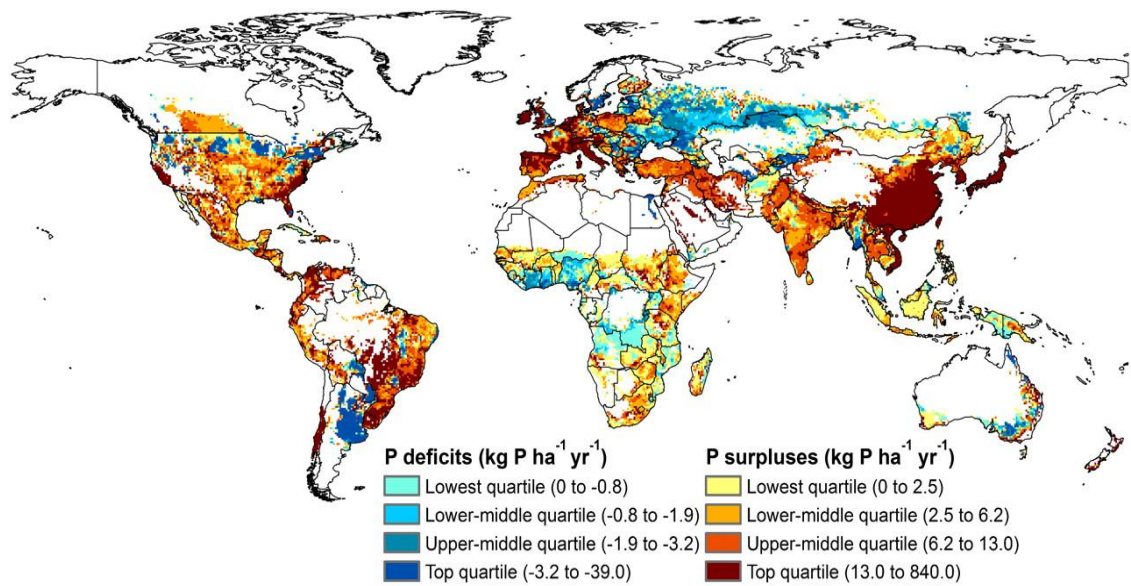


Fig 2.1 Global map showing agronomic P imbalances for the year 2000. The P surpluses and deficits are each classified according to quartiles globally (0–25th=lowest quartile, 25–50th =Lower middle quartile, 50–75th Upper middle quartile and 75–100th percentiles=Top quartile) Source: MacDonald *et al.*, 2011

The movement from the xylem to the cytoplasm and from the cytoplasm to vacuole requires energy for transport (Ullrich and Novacky, 1990). The *OsSPX-MFS/PHT* is responsible for transport of Pi into the vacuole and from the vacuole to different cellular compartments (Wang *et al.*, 2015; Liu *et al.*, 2016). The absorbed P is translocated from the older leaves to the younger leaves and loading of Pi into xylem for longer distance transport is mediated by gene like *PHO*.

Adequate nutrient supplied like P at early growth stages ensures adequate P for grain development because of its mobility within plants. If the soil is deficient in P, and if the plant suffers from P deficiency, it cannot sustain high yields because no plant can grow normally or give good yield (Tandon, 1987). However, plants have developed strategies to tolerate P deficiency and maximize P uptake using morphological and biochemical mechanism such as adoption of morphological changes in roots by producing more lateral roots and root hairs or increase in root biomass for better uptake of P from soil (Watnabe *et al.*, 2006; Lynch, 2011; Tyburski *et al.*, 2012; Niu *et al.*, 2013; White *et al.*, 2013; Florez-Sarasa *et al.*, 2014; Delgado *et al.*, 2015).

Secondly, plants can tolerate the P deficiency by using previously stored or assimilated Pi to be recycled. P recycling mechanism involving enzymes like phosphohydrolases, phosphatase, and ribonuclease can recycle, mobilize and translocate the organic P from older established tissues to younger and growing tissues (Yun and Kaeppler, 2001; Gong *et al.*, 2011; López-Arredondo *et al.*, 2014; Li *et al.*, 2015; Walder *et al.*, 2015; Liu *et al.*, 2016).

In some cases, to increase Pi uptake, plants take advantage of symbiotic associations with fungi (arbuscular mycorrhizal or ectomycorrhizal) that extend the root system of plants and make otherwise unavailable sources of organic P available for the plants (Smith and Smith, 2011). In symbiotic association, with the help of fungal Pi transporter, Pi is taken up into AM fungal hyphae which are then translocated to intracellular fungal structures in plant root cortical cells. Plant Pi transporters, transfer Pi from fungus to plant (Smith *et al.*, 2011).

2.5 Critical concentration level of P in rice plant

The critical concentration level of Pi or plant nutrient as a whole in rice plants is defined as the level in which deficiency symptoms or toxicity symptoms may become visible (De Datta, 1981). This critical concentration level of P in rice plants vary according to plant part that has been used for analysis and also crop age because of the mobility character of P in plants.

The concentration of P in the rice plant depends on plant variety, growing season, plant age and available P level in the soil (Marschner, 1995). The high percentage of P in seedlings decreases after transplanting, which then gradually increases and reach peaks at primodial initiation and again decreases after the flowering stage until the dough stage (Ishizuka *et al.*, 1965). This behavior is responsible for the mobility of phosphate within plants. Also, P absorbing power of the rice roots which is high during the vegetative and reproductive period is responsible for variation in P concentrations in rice plants (Marschner, 1995).

The concentration levels of nutrients like N and P influences each other through synergetic and antagonistic interaction between them especially when their level in the soil is at deficiency range (Marschner, 1990).

The concentration of P in most plants is between 0.1 and 0.4 % (Tisdale *et al.*, 1991). Plants whose concentration <0.1 % at tillering stage suffers from deficiency while plants with P concentration of >1 % suffers from P toxicity (Tanaka and Yoshida, 1970).

The critical concentration of Pi at maximum tillering stage in the rice plant is reported to be 0.1 %. And adequate P concentration in the rice plant as a whole is between 0.12 – 0.24 % (Mikkelsen, 1971). It is reported that P concentration of 0.21 % at panicle initiation stage in rice shoots associated with higher plant dry matter yield (Semoka and Shenkalwa, 1985)

2.6 Root architecture and P deficiency tolerance

Root architecture refers to the spatial configurations root system in response to environment *i.e.* soil conditions. It includes root topology, morphology and distribution patterns of roots. Concentration of P in the soil is one of the major factors that effects the root and its growth and morphology (Borch *et al.*, 1999; Williamson *et al.*, 2001)

The primary source of almost all the mineral elements required for plant growth is root and depending on the factors like water content, oxygen concentration, soil pH and nutrient availability *etc.*, the growth and development of roots vary greatly and shows plasticity (Vance *et al.*, 2003).

More number of root means more biomass of root that secret organic acid, phosphatases, and other substances that releases Pi from insoluble Pi sources (Hall, 1969; Pammenter and Woolhouse, 1975; Ae *et al.*, 1990; Otani *et al.*, 1996; Jain

et al., 2007; Liang *et al.*, 2010; Zhang *et al.*, 2010; Tian *et al.*, 2012; Zhu *et al.*, 2012; Lambers *et al.*, 2013).

Under low P condition, root architecture changes to adapt the starving situation by inhibiting primary root growth and inducing lateral root growth and root hairs development for better P acquisition from the soil (Niu *et al.*, 2013). Over expression of *OsMYB2P-1* in rice plant associated with shorter primary roots suggesting that *OsMYB2P-1* may have role in regulation of root system architecture (Dai *et al.*, 2012).

In the *ltn1* mutant line (*LTN1* the putative ortholog of Arabidopsis *PHO2*, which plays important roles in Pi starvation signaling), along with enhanced Pi uptake and transport, the elongation of primary and adventitious roots was enhanced under P deficiency condition, suggesting that *LTN1* is involved in Pi-dependent root architecture alteration (Hu *et al.*, 2011).

Rice plant growth model where rice plant were grown under highly P deficient condition to formulate hypotheses on pathways and genes causing genotypic differences in P uptake showed 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 (Wissuwa, 2003). The result also reported the changes in root architecture of rice grown under P deficiency condition with elongation of primary and adventitious roots. Changes in root architecture in response to low Pi are regulated mainly by sensing the local concentration of Pi in the soil solution.

Wasaki *et al.* (2003 a), reported that metabolic changes like secretion of acid phosphatase and organic acids from the root playing role in mobilization of phosphate from organic and inorganic substrates and active uptake of P from the rhizosphere. There is also increased production of ribonucleases which mobilizes Pi from internal RNA pools during P deficient condition (Li *et al.*, 2010).

Pectin in rice roots is found of playing role in reutilizing the immobilized P from cell wall in starved plants. In rice root, increased pectin content is found to be associating with increased level of soluble Pi during P starvation. It was proved that pectin release Pi from insoluble FePO₄ (Zhu *et al.*, 2015).

Knock-down of expression of NRR (nutrition response and root growth) by RNA interference resulted in enhanced rice root growth and over expression of NRR in rice exhibited significantly retarded root growth. Expression of NRR in rice seedling roots was significantly influenced by deficiency of macronutrients including P (Zhang *et al.*, 2012)

2.7 Identification of genes imparting P deficiency

To examine whether source or sink limitations were responsible for reduced root growth in P deficiency condition, an experiment was designed where rice plants were given four levels of P supply and two light treatments. The results showed that higher production of photosynthates under natural (high) light did not lead to additional growth under P deficiency suggesting that assimilate supply from source leaves to root was not a limiting factor under P deficiency for plant growth and development rather P deficiency was the key factor for poor growth and development (Wissuwa, 2003)

Numerous studies have been done to identify the genes/loci responsible for P deficiency tolerance. However, the molecular mechanisms that regulate gene expression during P starvation remain poorly understood; with only few transcription factors identified that each influence only a part of the spectrum of changes induced by P deficiency (Rouached *et al.*, 2010). Some of the important reported findings which are related to genes involved P starvation tolerance in rice are as follows.

Phosphorus uptake1 (*Pup1*) is a major QTL located on rice chromosome 12 that is associated with tolerance of phosphorus (P) deficiency in soil was first identified and characterized in the rice variety Kasalath (Wissuwa *et al.*, 1998; Wissuwa *et al.*, 2002). Mapping was done using NILs as a mapping population and the QTL *Pup1* was placed in a 3-Cm interval flanked by markers S14025 and S13126. The NILs carrying *Pup1* QTL showed significant yield difference compare to susceptible parent (Wissuwa, 2005; Chin *et al.*, 2010).

A gene was identified within *Pup1* QTL and the functional mechanism of this novel protein kinase-encoding gene (named P-Starvation TOLerance1, *OsPSTOL1*) has been characterized (Gamuyao *et al.*, 2012).

The over expression of *PSTOL1* in two transgenic rice varieties resulted in development of larger root system which improved the uptake of P and other nutrients, thereby enhanced the grain yield by 60 % under low P conditions (Chin *et al.*, 2011). And till date *Pup1* or more specifically *PSTOL1* is the only QTL which is being used under field conditions for low P tolerance.

Transcriptomic analysis showed significant induction of *OsPI1* (*Oryza sativa* phosphate-limitation inducible gene 1) under P deficiency condition. This gene

shares some of the properties with P deficiency-inducible gene family, *TPS11/Mt 4* and has been cloned and characterized (Wasaki *et al.*, 2003 b).

OsIPS1 identified as highly Pi-responsive found to be the sequester of miRNA 399, that prevents the miRNA 399 from binding to and cleavage of PHO2 mRNA, and the expression of PHO genes induces the further expression of Pi-responsive genes (Franco-Zorrilla *et al.*, 2007; Lin *et al.*, 2008)

Two *OsPHR* genes designated as *OsPHR1* and *OsPHR2* found to be playing role in Pi starvation signaling pathway by regulating the expression of Pi-starvation induced genes (Zhou *et al.*, 2008). Over-expression of *OsPHR2* results in the excessive accumulation of Pi in shoots under Pi-sufficient conditions and mimics as Pi-starvation stress resulting into proliferation of root hair growth and root elongation suggesting the involvement of *OsPHR2* in Pi-dependent root architecture. And also some Pi transporters were up-regulated under Pi-sufficient conditions, which correlates with the strongly increased content of Pi (Zhou *et al.*, 2008).

Over expression of *OsPHR* (*Oryza sativa* phosphate starvation response) which is a transcription factor with a MYB domain and functional ortholog gene of *AtPHR*, results in excessive accumulation of phosphate in rice shoots (Liu *et al.*, 2010). Knockout of *OsARF12* improved the absorption and translocation of Pi suggesting that *OsARF12* participated in *OsPHR2*-mediated _Pi response through controlling several -Pi response genes (Wang *et al* 2014a). In Arabidopsis and rice, *PHR1* is negatively regulated in a Pi-dependent manner by proteins containing SPX domain (Wang *et al.*, 2014b; Puga *et al.*, 2014; Wild *et al.*, 2016)

Characterization of root transcriptome under P deficiency condition showed differential expression of 5 genes (*OsMYB1*, *OsPHR1*, *OsPHR2*, *OsPHR3* and *OsPTI*) in F₂ population derived from cross between Sahbhagi Dhan and RRF-78 (Gupta *et al.*, 2016).

Several other genes involved in P transport, *OsPT 8* (*Oryza sativa* phosphate transporter) (Liu *et al.*, 2010; Jia *et al.*, 2011) and *OsPHF 1* (*Oryza sativa* phosphate transporter traffic facilitator) (Wang *et al.*, 2014a), signaling and regulatory mechanisms, *OsIPS1* and *OsIPS2* (*Oryza sativa* induced by phosphate starvation), *OsSPX1*, *OsSPX2*, *OsSPX3* (Liu *et al.*, 2010) and Pi homeostasis, *OsSPX 1-6* (Lopez-Arrendondo *et al.*, 2014) and *OsARF12* (Wang *et al.*, 2014a) have been identified in different rice cultivars and have been well reviewed by Heuer *et al.* (2017) and Das *et al.*, (2017).

Expression of *OsMYB2P-1* (localized in the nuclei and exhibited transcriptional activation activity) was induced by Pi starvation. Suppression of *OsMYB2P-1* using RNA interference showed transgenic rice more sensitive to P deficiency. Whereas, over-expression of *OsMYB2P-1* in rice as well as *Arabidopsis* enhanced tolerance to Pi starvation through greater expression of Pi responsive genes like *Oryza sativa* UDP-sulfoquinovose synthase, *OsIPS1*, *OsPAP10*, *OsmiR399a*, and *OsmiR399j* (Dai *et al.*, 2012). However, overexpression of *OsMYB2P-1* suppressed the expression of *OsPHO2* under both Pi-deficient and sufficient conditions, respectively (Dai *et al.*, 2012).

Similarly *OsMYB5P* increases tolerance to Pi deficiency in plants by modulating Pi transporters at the transcriptional level. Overexpression of *OsMYB5P* in rice and *Arabidopsis* (*Arabidopsis thaliana* Col-0) increases tolerance to phosphate starvation, whereas *OsMYB5P* knock-out through RNA interference increases sensitivity to Pi depletion in rice (Yang *et al.*, 2018).

Chen *et al.* (2015) identified the role of kinase subunit and holoenzyme, CK2 α 3/ β 3, which interacts with Phosphate Transporters (PTs). The activity of CK2 α 3/ β 3 was found to be regulated by P status and CK2 α 3/ β 3 was found to be negatively regulating the PTs.

Wasaki *et al.* (2003b) conducted a transcriptomic analysis using a cDNA microarray on rice and reported differential expression of many genes under P deficiency.

Genes putatively associated with root cell wall loosening and root hair extension (xyloglucan endotransglycosylases/hydrolases and NAD(P) H dependent oxidoreductase) showed higher expression in roots of tolerant NIL64 (Tanaka *et al.* 2009).

Wang *et al.* (2009) reported that five genes, *OsSPX1*, *OsSPX2*, *OsSPX3*, *OsSPX5* and *OsSPX 6* are responsive to Pi-starvation. *OsSPX1* and *OsSPX2* are located in nucleus, *OsSPX3* in the cytoplasm, and *OsSPX4* is a membrane localization protein. They also reported that *OsSPX1* regulates *OsSPX2*, *OsSPX3* and *OsSPX 5* at the transcription level and is positively involved in the responses of the genes to Pi starvation. *OsSPX3* is reported to negatively regulating genes like *OsSPX5* and *OsIPS1* (Pi-starvation induced) in shoots under Pi-sufficient condition and is involved in the responses of *miR399* and *OsPHO2* to Pi-starvation.

Post-transcriptional regulation of PT1 and other downstream Pi Starvation Induced (PSI) genes through *OsPHF1* reported to have potential application

for uptake, translocation and remobilization of Pi through the functions of Pi transporters (PTs) (Wu *et al.*, 2013). They also reported that the expressions of these PSI genes are regulated by OsPHR2 mediated transcriptional control and PHO2-mediated ubiquitination. Whereas, SPX domain containing proteins and the type I H⁺-PPase AVP1 is involved in the maintenance and utilization of the internal phosphate.

The 'OsCYCP1;1' protein which is predominantly localized in the nucleus and ubiquitously expressed throughout the whole plant during the entire growth period of rice was able to partially rescue the pho80 mutant strain of yeast. Findings suggested that, over expression and suppression *OsCYCP1;1* influenced the phosphate starvation signaling response and *OsCYCP1;1* expression was reduced by phosphate starvation in the roots (Deng *et al.*, 2014).

LTN1 (LOC_Os05g48390) mutant (*ltn1*), the putative ortholog of Arabidopsis PHO2, which plays important roles in Pi starvation signaling, exhibited increased Pi uptake and translocation, which led to Pi over accumulation in shoots (Hu *et al.*, 2011). Plant over expressing *OsmiR399* showed down-regulation of LTN1, which strongly indicate that LTN1 is involved in crucial Pi starvation signaling pathway downstream of *miR399* in rice (Hu *et al.*, 2011).

2.8 Screening of genotypes for low P tolerance

Developing rice cultivars with an improved tolerance to P deficiency is the sustainable to tackle the problem of low productivity of rice due to low P. therefore, the screening and identification of genotypes carrying genes/QTL that that can be use for introgressing into the desired genotype for better crop production.

P deficiency tolerant cultivar IR20 is crossed with IR551783B9-3 (sensitive to P-deficiency) to generate RILs and through AFLP analysis and mapping, a major QTL (designated as PHO) located on ch # 12 for P deficiency tolerance was identified (Ni *et al.*, 1998).

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

Diverse rice genotypes adapted to low P acidic soil were evaluated for nucleotide variation in two reported loci for low P tolerance, namely *PSTOL1* and *PupK20-2*. The results led to the discovery of novel SNPs across both *PSTOL1* and

PupK20-2 suggesting that there is role of novel haplotypes in in adaptation to low land acidic condition (Yumnam *et al.*, 2015).

2.9 Hormones in P starvation tolerance

Strigolactone (SLs) are plant hormones and may have important physiological roles during growth and development of plants (Koltai, 2011) especially during nutrient deficiency like P because its biosynthesis is promoted during low Pi conditions (Umehara *et al.* 2010). SLs reported to have effect on root architecture which is mediated by auxin (Ruyter-Spira *et al.* 2011). SL has direct effect on the induction and elongation of crown roots in rice during Pi starvation (Arite *et al.* 2012) and as report large root surface area is essential for better uptake of the nutrients from the soil (Lynch, 2007). It is also found that SLs are secreted from roots into the rhizosphere where they stimulate symbiosis with fungi (arbuscular mycorrhizal) which ultimatelt leads to better uptake of Pi from soil (Xie & Yoneyama, 2010; Umehara *et al.* 2010). However, SLs is reported to inhibit tillering or shoot branching by suppressing axillary bud outgrowth (Jamil *et al.* 2012).

The primary root growth halt influences the change in concentration of the plant hormone Auxin and lateral root growth may be induced due to higher concentration of auxin (Nacry *et al.*, 2005).

ABA is a plant stress hormone and derived from the carotenoid pathway similar to SLs and it is assumed that they interact with each other as it was found in ABA deficient tomato mutant has reduced level of SLs too (López-Ráez *et al.* 2010).

ABA hormones play an important role during P starvation as chemical messengers for molecular, biochemical and physiological regulation underlying growth and development of plant (Alcázar *et al.*, 2010).

ABA also influences root growth and morphology and shoot/root ratios during nutrient stress condition like phosphate deficiency. Therefore, the application of ABA can be done to positively influence root growth and development of rice under low condition (Chen *et al.*, 2006).

Ethylene positively regulates pectin content and expression of *OsPT2*, which ultimately makes more P available by facilitating the solubilization of P fixed in the cell wall and its translocation to the shoot (Zhu *et al.*, 2016). ACC (1-amino-cyclopropane-1-carboxylic acid, an ethylene precursor reported to be positively regulating pectin content and expression of *OsPT2*, which ultimately leads to more P

available by inducing the solubilization of P fixed in the cell wall and its translocation to the shoot (Zhu *et al.*, 2016).

2.10 G2-like transcription factor

G2 stands for golden 2 gene family which was first identified after the isolation of a pale green maize mutant that exhibited perturbed development of photosynthetic cell types. The name, *Golden2*, nomenclature was used for the first golden producing factor, *golden1*, found in maize by Jenkins (1926).

G2 gene regulates the plastid biogenesis in all photosynthetic cells and plays a critical role in the development of chloroplasts in C3 mesophyll and C4 bundle sheath cells (Langdale and Kidner, 1994). Sequence analysis suggested that G2 acts as a transcription factor (Hall *et al.*, 1998).

Similar to G2 genes, Golden 2-like transcription factors (GLK) are involved in chloroplast development and have been reported to co-regulate and adjust the expression of a set of nuclear photosynthetic genes and thus act to optimize photosynthetic capacity in different developmental and environmental conditions (Waters *et al.*, 2009).

Golden2-like (GLK) transcription factors belongs to the members of the GARP family of Myb transcription factors with an established relationship to chloroplast development in the plant kingdom (Chen *et al.*, 2016). The *Oryza sativa* G2-like transcription factor family has 62 members in *O. sativa*, *Japonica* type and 52 members in *O. sativa*, *Indica* type (PlantTFDB), but the roles of most of GLK members are still uncharacterized. Most G2-like genes have two domains, a Myb-DNA binding domain (DBD; containing an HLH motif) and a C-terminal domain (containing a conserved GCT box).

Being the members of the GARP superfamily of transcription factors, it has diverse functions such as nutrient sensing, root and shoots development, floral transition, chloroplast development, circadian clock oscillation maintenance, hormonal transport and signaling (Safi *et al.*, 2017). The GLKs and its phylogenetic analysis demonstrated that the maize, rice and Arabidopsis GLK gene pairs comprise a distinct group within the GARP superfamily. Phylogenetic analysis also showed that the gene pairs arose through separate duplication events in the monocot and dicot lineages (Fittter *et al.*, 2002).

The *Golden2* (G2) gene function specifically for the differentiation of bundle sheath cell chloroplasts in C4 leaf blades. However, expression of GLK genes

suggest that these genes may act redundantly to promote photosynthetic development in this C3 species. Whereas, in maize, *G2* and *ZmGlk1* transcripts accumulate primarily in C4 bundle sheath and C4 mesophyll cells, respectively, suggesting a specific role for each gene in C4 differentiation. The *G2* and *ZmGLK1* act as transcriptional regulators of cell-type differentiation processes (Rossini *et al.*, 2001).

G2 plays a critical role in cellular differentiation (Hall *et al.*, 1998); and expression of GLKs in maize, rice and *Arabidopsis* is regulated by light (Rossini *et al.*, 2001; Fitter *et al.*, 2002), while *GLKs* in *Brassica* spp. are induced by cold stress (Savitch *et al.*, 2005).

G2 is expressed in bundle sheath cells, whereas its homologue *ZmGLK1* is expressed most strongly in mesophyll cells (Rossini *et al.*, 2001). *G2* and *ZmGLK1* each has a central function in the series of events that leads to bundle sheath and mesophyll cell differentiation (Waters and Langdale, 2009).

The MYB gene family comprises one of the richest groups of transcription factors in plants. Plant MYB proteins are characterized by a highly conserved MYB DNA-binding domain. MYB proteins are classified into four major groups namely, 1R-MYB, 2R-MYB, 3R-MYB and 4R-MYB based on the number and position of MYB repeats. MYB transcription factors are involved in plant development, secondary metabolism, hormone signal transduction, disease resistance and abiotic stress tolerance (Katiyar *et al.*, 2012).

In maize which is a C4 plant, the two GLK genes has compartmentalized function in bundle sheath and mesophyll cells which regulates dimorphic chloroplast differentiation, whereas in the C3 plants *Physcomitrella patens* and *Arabidopsis thaliana* the genes act redundantly in all photosynthetic cells (Wang *et al.*, 2013).

2.11 Role of G2-like transcription factor in plants

Plant specific GARP (Golden2, ARR-B, Psr1) transcription factor family contains genes involved in: root and shoot development, floral transition, chloroplast development, hormonal transport and signaling, organites biogenesis, circadian clock oscillations, resistance to pathogens, as well as genes that may constitute a central nutrient sensing system in plants (Safi *et al.*, 2017). GARP is named after maize GOLDEN2 (*G2*), the ARR B-class proteins from *Arabidopsis*, and *Chlamydomonas* Psr1 (Riechmann *et al.*, 2000).

Arabidopsis thaliana *AtGLK1* and *AtGLK2* genes have been shown to act redundantly to regulate chloroplast development (Fitter *et al.*, 2002; Yasumura *et al.*, 2005). *AtGLKs* have shown to bind to promoters of several photosynthetic genes to upregulate the transcription of chloroplast genes (Waters *et al.*, 2009). In addition to binding to promoters, *AtGLK1* also interacts with proline-rich activation domains of G-BOX binding bZIP factors, suggesting that *GLKs* may regulate other related plant processes (Tamai *et al.*, 2002). *GLK1* (*AtGLK1*, *GPRI1*, *At2g20570*) and *GLK2* (*AtGLK2*, *GPRI2*, *At5g44190*) are similar to type *B ARABIDOPSIS RESPONSE REGULATORS* (*ARRs*), which are families of plant proteins related to prokaryotic response regulators of two-component systems (Riechmann *et al.*, 2000). *GLK* directly binds to the promoters of target genes and activate the transcription of target genes (Waters *et al.*, 2009). The loss-of function *glk1 glk2* double mutant results in pale green in all photosynthetic tissues, with fewer thylakoid grana, whereas the loss of either *AtGLK1* or *AtGLK2* alone does not produce a pale green phenotype (Fitter *et al.*, 2002). The *AtGLK* proteins act in a cell autonomous manner to coordinate and maintain the photosynthetic apparatus within individual cells, and help to co-regulate and synchronize the expression of nuclear photosynthetic genes to optimize photosynthetic capacity in varying environmental and developmental conditions (Waters *et al.*, 2009).

GLK transcription factors present in the nuclear genome are involved in the related expression of nuclear chloroplast-localized proteins and photosynthesis-related genes in maize (*Zea mays*), the moss *Physcomitrella patens* and *Arabidopsis thaliana* (Hall *et al.*, 1998; Rossini *et al.*, 2001; Fitter *et al.*, 2002; Yasumura *et al.*, 2005; Waters *et al.*, 2009).

GLKs-MYB transcription factors are the major drivers of photosynthetic genes, with one (*GLK2*) being light-induced (Jarvis and Juez, 2013). Two types of transcription factors, *LONG HYPOCOTYL5* (*HY5*) and *GLKs*, are involved in the regulation of root greening; the former is a pivotal factor for chlorophyll accumulation in roots and the latter is a strong activator of root greening. The combination of at least these two transcription factors is involved in the coordinated expression of nuclear-encoded photosynthesis related genes in the root (Kobayashi *et al.*, 2012).

Waters *et al.* (2009) reported that potent transcription factors, *GLK1* and *GLK2*, coordinate the expression of nuclear-encoded photosynthesis-related genes, including chlorophyll biosynthesis genes in *Arabidopsis*. Consistent with the fact that *GLK2* transcript is more abundant than that of *GLK1* in the root (Fitter *et al.*, 2002).

GLK2 may be responsible for regulation of the light-harvesting genes during Pi starvation in the wild-type (Kobayashi *et al.*, 2013). The over expression of *GLK* enhances the expression of genes related to fruit photosynthesis and chloroplast development, leading to elevated carbohydrate and carotenoid levels in ripe fruit. Also, *SIGLK2* influences photosynthesis in developing fruit, contributing to mature fruit characteristics (Powell *et al.*, 2012)

In *glk1* and *glk2* mutants several genes encoding proteins associated with light harvesting and chlorophyll biosynthesis are downregulated (Fitter *et al.*, 2002; Yasumura *et al.*, 2005). However, over expression of *GLK* genes led to transcript levels that were approximately two to three fold higher than the wild type. This indicated that *GLK* genes promote the transcription of genes responsible for key steps in chlorophyll biosynthesis (Waters *et al.*, 2009).

ZmGLK1 is thought to regulate mesophyll cell chloroplast development in C₄ tissues, and *GLK* gene pairs act redundantly to promote chloroplast development in C₃ species (Waters and Langdale, 2009; Fitter *et al.*, 2002).

In tomato (*Solanum lycopersicum*) fruit, the *uniform ripening (u)* locus, which encodes a *GOLDEN2-LIKE* transcription factor (*SIGLK2*), influences a gradient of chloroplast development that extends from the stem end of the fruit surrounding the calyx to the base of the fruit (Nadakuduti *et al.*, 2014).

Similarly, over-expression of *ARABIDOPSIS PSEUDO RESPONSE REGULATOR 2-LIKE (SIAPRR2-LIKE)* in tomato, which is related to *SIGLK2*, causes enhanced development of fruit chloroplasts (Pan *et al.*, 2013).

Nakamaru *et al.* (2009) strongly suggested that *OsGLK1* regulates chloroplast development under the control of light and phytohormones, and that it is a key regulator of chloroplast development.

Tomato (*Solanum lycopersicum*), like most plants, contains two *GLKs* (i.e., *GLK1* and *GLK2/UNIFORM*), mutant and transgene analysis demonstrated that these genes encode functionally similar peptides, though differential expression renders *GLK1* more important in leaves, while *GLK2* is predominant in fruit (Nguyen *et al.*, 2014).

Phytochrome-interacting factor 4 (*PIF4*) acts as a transcriptional activator that up-regulates the expression of regulator *NYE* (Non-yellowing1) that triggers the degradation of chlorophyll and it acts as a transcriptional repressor for

PORC and *GLK2* to inhibit the chlorophyll biosynthesis and chloroplast activity which ultimately results in early leaf senescence (Song *et al.*, 2014).

In Arabidopsis, a core organellar gene expression (OGE) response module was defined by identifying genes that were differentially expressed under different conditions relevant to OGE signaling. The result identified over expressors of the *GLK1* and *GLK2* as *genomes uncoupled (gun)* mutants (Leister and Kleine, 2016).

Transgenic analysis in tomato has shown that over expressing *SIGLK2* gene confers dark green fruits with high chlorophyll and chloroplast levels in addition to a more stacked thylakoid grana and elevated starch in the fruit (Powell *et al.*, 2012; Nguyen *et al.*, 2014). Molecular evidence showed that the tomato *GLK2* protein is a substrate of the CUL4 (CULLIN4)-DDB1 (DNA BINDING PROTEIN1)-DET1(DE-ETIOLATED1) ubiquitin ligase complex for the proteasome degradation (Tang *et al.*, 2016).

The promoter region of the tomato *GLK1* contains conserved ARF binding sites (TGTCTC box) suggesting that *ARF* might have role in regulating expression of *GLK1* gene in tomato. Down regulation of *ARF4* expression resulted in up-regulation of *GLK1* gene which in turn increases the accumulation of chlorophyll (Sagar *et al.*, 2013)

In rice, the *GLK* plays a critical role in regulating flowering time. *OsPHL3* belongs to *GLK* containing MYB-CC domain, identified as a new gene involved in regulating the flowering pathway in rice that acts as a constitutive repressor of flowering under both long day (LD) and short day (SD) duration. Over expression of *OsPHL3* delayed flowering time in rice under LD and SD conditions. Knockout of *OsPHL3* by genome-editing tool CRISPR/Cas9 resulted in earlier flowering regardless of day-length (Zeng *et al.*, 2018).

2.12 Role of G2-like transcription factor in stress tolerance

Liu *et al.* (2016) identified 59 G2-like genes in B73 maize genome. Based on gene structure character, motif compositions and phylogenetic analysis, maize G2-like genes (ZmG1- ZmG59) were divided into seven groups (I-VII). By synteny analysis, 18 collinear gene pairs and strongly conserved microsyntny among regions hosting G2-like genes across maize and sorghum were found. The *GLK* were highly conserved during evolution. They concluded that *GLK* genes might be related to cold and drought stresses. It plays a role in disease defense and their function is conserved in regulating chloroplast development (Chen *et al.*, 2016). *GLK* proteins act

as key photosynthetic regulators as part of plant acclimation to variable environmental circumstances (Waters and Langdale, 2009).

The effects of over expression of two Brassica CBF/DREB1-like transcription factors (BNCBF5 and 17) in *Brassica napus* cv. resulted in the development of constitutive freezing tolerance and accumulation of COR gene mRNAs, BNCBF5- and 17-overexpressing plants along with moderate accumulation of transcript levels genes (*GLK1* and *GLK2*) involved in photosynthesis and chloroplast development (Savitch *et al.*, 2005). Report suggested that *GLK* genes play a role in the temperature stress response, as increased accumulation of *GLK1* was observed in frost-tolerant transgenic *Brassica napus* overexpressing two transcripts harboring DREB1/CBFs (Savitch *et al.*, 2005).

In Arabidopsis, *GLK* genes are reported to play role in defense related mechanisms (Ryals *et al.*, 1996; Kunkel and Brooks, 2002; Savitch *et al.*, 2007; Nakamura *et al.*, 2009; Jhadeswar *et al.*, 2014; Han *et al.*, 2016). The overexpression of *AtGLK1* has shown to play a role in pathogen response (Savitch *et al.*, 2007), in nitrogen use efficiency (Gutiérrez *et al.*, 2008), in light- and brassinosteroid-induced chloroplast development (Nakamura *et al.*, 2009; Yu *et al.*, 2011) and in root hormonal signalling (Kobayashi *et al.*, 2012).

Constitutive overexpression of *AtGLK1* (35S:*AtGLK1*) confers enhanced resistance to *Fusarium graminearum*, a non host fungal pathogen in *Arabidopsis* (Savitch *et al.*, 2007; Schreiber *et al.*, 2011), suggesting that *AtGLK1* may play a role in plant defence (Mukhtar *et al.*, 2012).

The *AtGLKs* can also interact with ANAC092 to modulate senescence (Rauf *et al.*, 2013), involvement of ANAC092 in jasmonic acid (JA)/ethylene (ET) signalling (Bu *et al.*, 2008) and in disease resistance (Al-Daoud and Cameron, 2011). It is possible that *GLKs* may also be involved in responses to pathogen attack.

Murmu *et al.* (2014) suggested that *AtGLK* mediated plant defence to *Hyaloperonospora arabidopsidis* (*Hpa*) Noco2 was partially dependent on salicylic acid (SA) accumulation, but independent of the SA signaling protein *NON EXPRESSOR OF PATHOGENESIS-RELATED 1* (*NPR1*). They concluded that *GLK* acts upstream of JA signalling in disease susceptibility to *Hpa* Noco2. Also *AtGLK1* is involved in jasmonic acid (JA)-dependent susceptibility to the biotrophic pathogen *Hpa* Noco2 and in JA-independent resistance to the necrotrophic pathogen *Botrytis cinerea*.

In paper mulberry, expression of fifteen *GLK* genes were induced and *GLK* genes were repressed when exposed to cold stress which suggest that *GLK* genes may be playing some role under abiotic stresses such as cold, etc (Peng *et al.*, 2015).

Nagatoshi *et al.* (2016) used chimeric repressor gene-silencing technology (CRES-T) and identified the function of *Arabidopsis GLK1* and *GLK2*, as positive regulators of stomatal movement and K^{+}_{in} channel genes. The chimeric *GLK* repressors induce closed stomata and tolerance to ozone exposure. Hence, guard cell-specific expression of this repressor may be a useful tool to confer resistance to air pollutants.

Petridish *et al.* (2016) identified novel transcription factors involved in the regulation of phenylpropanoid metabolism during cold acclimation. Two genes *Arabidopsis thaliana G2-LIKE FLAVONOID REGULATOR (GFR)* and *BRASSINOSTEROID ENHANCED EXPRESSION1 (BEE1)* act as low-temperature regulators of flavonoid accumulation under low temperature stress condition suggesting that *GLK* might have role in cold stress tolerance.

Arabidopsis glk1-glk2 double-mutant was found to be susceptible to CMV infection and suffered from damages like higher oxidative damages, more compromised in PSII photochemistry and more reactive oxygen species accumulation. The result suggested that *GLK1* and *GLK2* might have function in virus resistance (Han *et al.*, 2016).

Chapter 3

Materials and Methods

The current study was conducted to understand the phosphorus (P) deficiency tolerance mechanism in rice plant. The work involved a) advancement of a biparental mapping population, b) marker trait association and c) evaluation of the role of one *Oryza sativa* transcription factor family (*i.e.* *OsGLK*) in P deficiency tolerance using *in-silico* tools and real time PCR. For this purpose, two diverse rice genotypes namely Sahbhagi Dhan (LR 23) and Chakhao Poreiton (LR 26) were selected for generating the mapping population. Sahbhagi Dhan (LR 23) (IR74371-70-1-1) is a conventionally bred, drought-tolerant rice variety identified in 2009 by the Central Variety Release Committee (CVRC) and released for cultivation in India, in 2010. Whereas, Chakhao Poreiton (LR 26) is the local sticky aromatic black rice of Manipur, India. Both SD (LR 23) and CP (LR 26) are tolerant to P deficiency under acidic condition, but only SD (LR 23) has *PSTOL1* gene which is absent in CP (LR 26), suggesting that there are other molecular mechanisms involved during P starvation which is providing ability to CP (LR 26) to tolerate low P under acidic condition (Yumnam *et al.*, 2015).

➤ Rationale for selecting GLK gene family was as follows:

1. It is a transcription factor family, therefore has a wide range of functions including regulation of genes related to P starvation signaling and tolerance.
2. Important for chloroplast synthesis.
3. Its members have been reported to co-regulate and adjust the expression of a set of nuclear photosynthetic genes and thus act to optimize photosynthetic capacity in different developmental and environmental conditions.
4. Root transcriptome data suggested the role of some members of *OsGLK* in P deficiency tolerance (Tyagi and Rai, 2017).
5. GLK members have been reported to play a role in many abiotic stress tolerance like frost and temperature stresses (Savitch *et al.*, 2005), response against pathogens (Murmu *et al.*, 2014) and in regulating fruit maturity (Nadakuduti *et al.*, 2014) and days to flowering (Zeng *et al.*, 2018).

3.1 Description of the study area

The study was conducted in lab and plant breeding field (Fig. 3.2) of College of Post Graduate Studies in Agricultural Sciences, Umiam, Meghalaya, situated at 25° 41' latitude and 91° 54' longitude and an elevation of 950 m above mean sea level. The climatic condition of the region as a whole is sub-tropical, humid with hot summer and cold winter, the average annual temperature of Umiam, Meghalaya, varies from 4° C in winter and 32° C in summer months respectively. Plants were grown during the Kharif season and as a rainfed crop. The soil pH at the time of rice growing season was recorded to be between 5.10-5.5.

3.2 Generation of mapping population

A cross (F_4 generation) between genotype LR 23 (PSTOL1⁺) and LR 26 (PSTOL1⁻) already available in the lab was used for further advancement in the rice growing season of 2015, 2016 and 2017 as shown in Fig 3.1. From F_4 generation onwards, in each generation, the seeds were sown in the nursery bed and the plant populations were transplanted to the field after 25-30 days of sowing following augmented design. The transplanting was done by maintaining spacing of 20 cm row to row and 20 cm plant to plant. Two parental lines and three other genotypes namely LR 55, CAUR1 and LR 60 were transplanted in each plot as a check/control. Detailed process involved in generation of RILs is provided in Fig. 3.1 and Fig. 3.2.

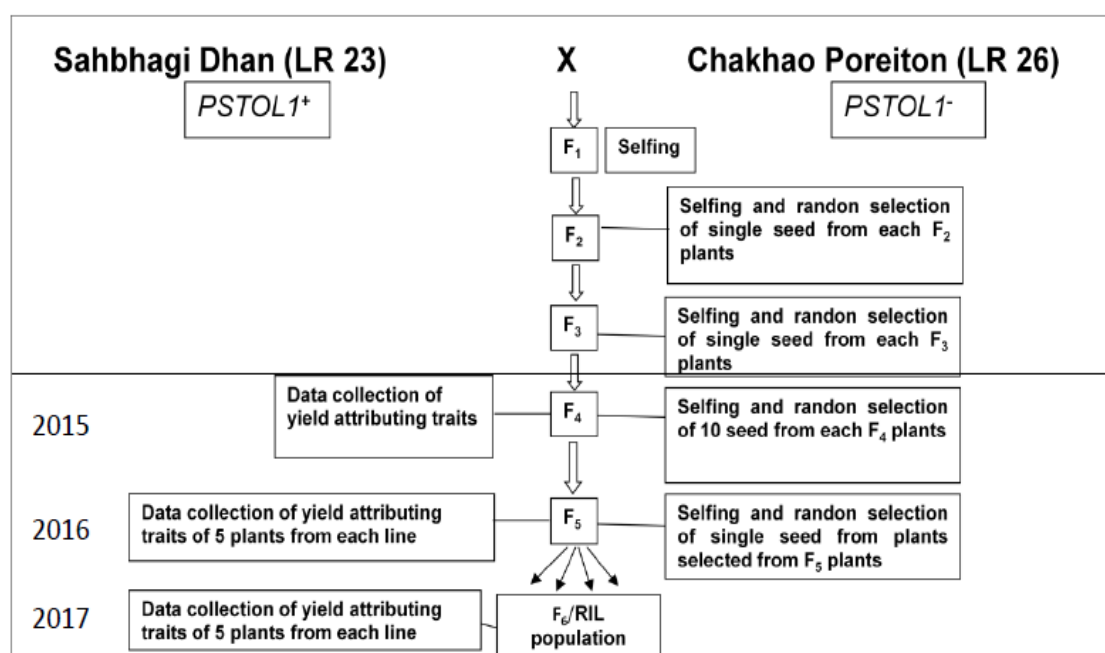


Fig. 3.1 Generation of RILs mapping population



Fig. 3.2 (A) Field preparation and transplanting of rice seedlings (B) Field view after 40 days of transplanting.

3.3 *In silico* analysis of OsGLK transcription factor family

In silico analysis of G2-like transcription factor was done by using different available online databases. The information regarding number of members, nucleotide and amino acids sequence, phylogenetic analysis, putative function of the proteins, different protein domains, *cis*-acting elements present in 5' UTR (Untranslated Region) of the genes, etc were acquired from different databases and are discussed in detail below.

3.3.1 Retrieving nucleotide and amino acid sequences of OsGLK members

The number and details of members of OsGLK family members belonging to rice were obtained from Plant transcription factor database (PlantTFDB) (<http://planttfdb.cbi.pku.edu.cn>) (Guo *et al.*, 2008). *Oryza sativa* have 62 members of OsGLK family in *japonica* type and 52 members in *indica* type. For further analysis of the OsGLK members, splice variants were excluded. The genomic nucleotide sequence information of each member was obtained from Rice Genome Project Annotation database (<http://rice.plantbiology.msu.edu/>) using genome browser tool. The CDS (Coding DNA Sequence) and amino acid sequences of each member were retrieved from plant transcription database (PlantTFDB) (<http://planttfdb.cbi.pku.edu.cn>). Rice Genome Project Annotation database (<http://rice.plantbiology.msu.edu/>) was also used to retrieve the 1 kb 5'UTR DNA sequences of each OsGLK members. The obtained DNA and amino acids sequences were further used for designing RT (Reverse Transcription) primers as well as candidate gene based primers and for phylogenic analysis, respectively.

3.3.2 Retrieving basic information and putative functions of OsGLKs

The basic information like length of CDS and amino acid sequence, genomic position, tissue-specific expression pattern, disruption phenotype, induction, biological and molecular putative function of OsGLKs were obtained from Plant transcription factor database (PlantTFDB) (<http://planttfdb.cbi.pku.edu.cn>) and Rice Genome Project Annotation database (<http://rice.plantbiology.msu.edu/>). The protein annotations were also obtained from Rice Genome Project Annotation database (<http://rice.plantbiology.msu.edu/>).

3.3.3 OsGLKs maize orthologs and mutant identification

The maize ortholog of each member of OsGLKs was identified/obtained from Plant transcription factor database (PlantTFDB)

(<http://planttfdb.cbi.pku.edu.cn>) and Rice Genome Project Annotation database (<http://rice.plantbiology.msu.edu/>). The identity of rice mutant lines available were retrieved from RiceGE: Rice Functional Genomic Express Database (<http://signal.salk.edu/cgi-bin/RiceGE?gene>). And from the same database *i.e.* RiceGE: Rice Functional Genomic Express Database (<http://signal.salk.edu/cgi-bin/RiceGE?gene>), the orientation of the gene in the genome was obtained.

3.3.4 Identification of *Cis*-acting elements

Genomic sequences from 5' UTR (1 Kb upstream region from starting nucleotide of CDS) were first retrieved from Rice Genome Project Annotation database (<http://rice.plantbiology.msu.edu/>). The obtained sequences were then used to identify the available *cis*-acting elements within those 1 Kb regions. For this, 1 kb 5' UTR sequences were converted to fasta format using online sequence format converter tool (<http://avermitilis.ls.kitasato-u.ac.jp/readseq.cgi>) and used for further analysis. The information regarding number and functions of *cis*-acting elements present in each member of *OsGLKs* were obtained using online database called PLACE (<http://www.dna.affrc.go.jp/htdocs/PLACE/>). It is a database of motifs found in plant *cis*-acting regulatory DNA elements, all from previously published reports (Higo *et al.*, 1998).

3.4 Amino acid frequency in members of *OsGLKs* and identification of protein domains

The frequency distribution of different amino acids in each member was calculated using sequence alignment tool available in MEGA software (<https://www.megasoftware.net>). The frequency distribution was calculated in term of percentage. With the help of Plant transcription factor database (PlantTFDB) (<http://planttfdb.cbi.pku.edu.cn>), major protein domain and its location within the polypeptide chain were identified.

3.5 Phylogenetic analysis of *OsGLK* members using protein sequences

After obtaining protein sequences of each member of *OsGLK* family, the sequences were subjected to phylogenetic analysis. The amino acid sequences were first converted to FASTA format using format converter online tool. The FASTA format protein sequences were then used for multiple sequence alignment and creation of phylogenetic tree for understanding the similarities and differences and

distance within the members of *OsGLK* family. The sequence alignment and phylogenetic analysis was done by using MEGA software (MEGA 5.2) (<https://www.megasoftware.net>). The phylogenetic tree was constructed using neighbor joining method available in MEGA 5.2 software with 1000 bootstrapping value.

3.6 Hydroponic screening

The two parental rice lines (LR 23 and LR 26) both tolerant to low P conditions and two susceptible genotypes, namely; BAM 350 and BAM 811 (unpublished lab data) were selected for expression study. This was done using hydroponic screening for low P condition followed by RNA isolation. Around 20 seeds of each genotype were germinated on moist germination paper which was placed in petri plates in greenhouse conditions (temperature of 24-28 °C and about 70 % relative humidity). The germinated 2-3 cm long seedlings were then transferred to disposable cups (Fig. 3.3) (with thermocol punctured with holes and fixed with net for support) containing modified Yoshida solution (Table 3.1) and grown for 7-8 days. The solution was changed after every 3 days. After 7-8 days of growing, seedlings were divided into two sets of 10 plants each. One set was grown under optimal P condition (0.31 mM) and another set of 10 in low P conditions (0.015 mM) for 24 hours. After treatment time, shoots were harvested and immediately dipped into the liquid nitrogen. The frozen sample were wrapped with aluminum foil and stored in -80 °C for further work.

Table 3.1. Composition of modified Yoshida solution used for hydroponic screening

Solution	Chemical names	Concentration	Amount (g/l)
1	Ammonium sulfate	0.7 M	92.498
2	Potassium nitrate	1.0 M	101.10
3	Sodium dihydrogen phosphate	0.35 mM	35.60
4	Calcium chloride, dihydrate	0.015 mM	117.35
5	Magnesium sulfate, heptahydrate	1.3 M	324
6	Manganous chloride, tetrahydrate	9.0 mM	1.50
	Ammonium molybdate, tetrahydrate	0.05 mM	0.074
	Zinc sulfate, heptahydrate	0.1 mM	0.035
	Boric acid	0.01 M	0.934
	Cupric sulfate, pentahydrate	0.1 mM	0.031
	Ferric chloride, hexahydrate	0.02 M	7.70
	Citric acid, monohydrate	0.05 mM	11.90

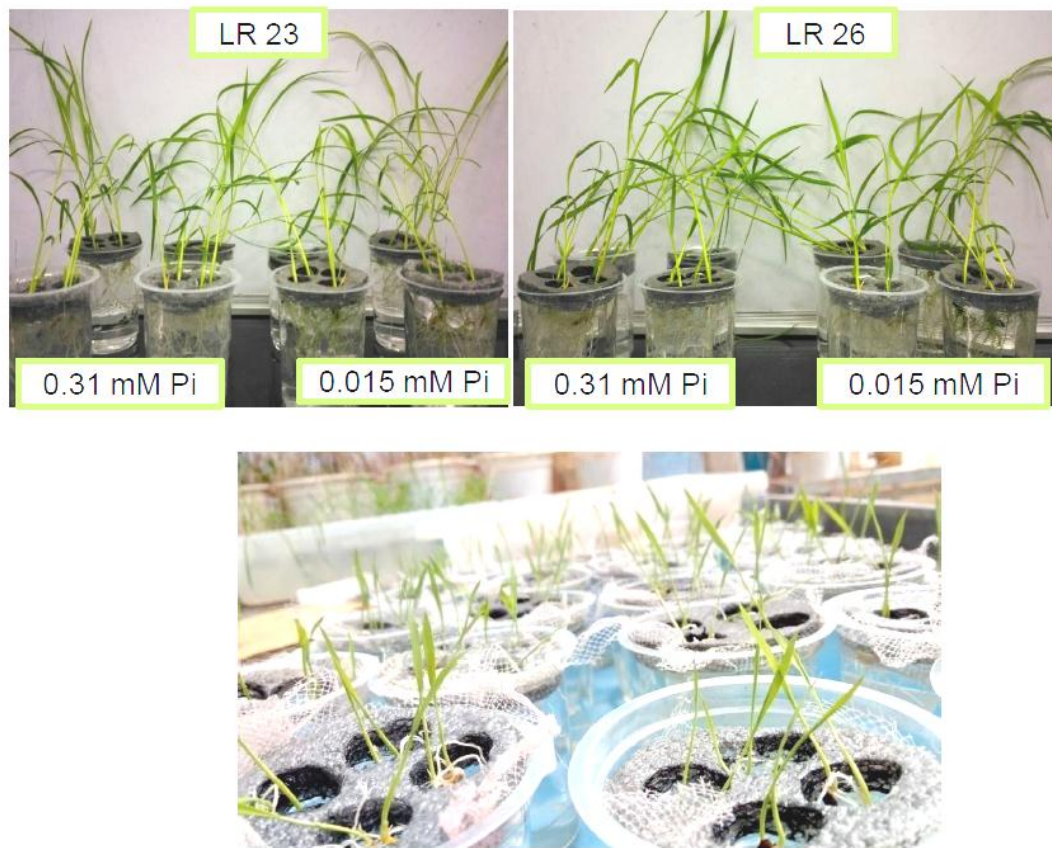


Fig. 3.3 Hydroponic screening of two parental genotypes

3.7 Total RNA extraction

Total RNA extraction from both control and treated frozen leaf samples was done by using Spectrum™ Plant Total RNA Kit (Sigma-Aldrich). First strand complementary DNA (cDNA) from total mRNA extracted was synthesis by reverse transcription. For reverse transcription, 5 µl RNA (~200 µg), 400 µM oligo-d(T)₂₃ (New England Biolabs Inc. #513275), 0.5M DTT (Sigma), 5X buffer (Thermo Scientific), 10mM dNTPs (Thermo Scientific, #R0192), 200 U of reverse transcriptase enzyme (Thermo Scientific) were used. Total reaction volume of 50 µl was made by adding PCR grade water. The cDNA synthesized, was then used for semi quantitative and real time PCR using *OsGLK* member specific RT primers.

3.8 RT primer designing

RT-PCR primers specific to 45 members of *OsGLK* family members excluding the splice variants were designed using free online software Primer3 (http://biotools.umassd.edu/bioapps/primer3_www.cgi). The forward and the reverse RT-PCR primers were designed targeting the coding sequence of the genes with an amplicon size of about 100 bp (Table 3.2). The primers were sent to Eurofins Genomics India Pvt. Ltd. for synthesis. The lyophilized primers were dissolved in 1X TE and working solution of 10 µM was prepared. The PCR conditions for RT primers were first standardized on parental genomic DNA and only those primers giving single sharp band were used for semi-quantitative PCR.

Table 3.2 Sequence list of RT primers for 45 OsGLK members with amplicon size

Sl. No.	Name of Primer	Forward (5'-3')	Reverse (5'-3')	Expected amplicon size (bp)
1	CAU-G2-RT01	GACGACAACGACGAGATCAA	ACGCGTGGTGATTTGATTG	97
2	CAU-G2-RT02	CATGTCTGAGCTGCACAAGC	GAGGAGACGATCGGTGTGAT	96
3	CAU-G2-RT03	GATTCCCCTCCTTCCAGAAC	TTGGCTATCTTTTGGGGAAA	86
4	CAU-G2-RT04	CTTTGCATCATCCTGAGCTG	TGGGAACATACCAAAGAATCATC	81
5	CAU-G2-RT05	CCACCACTCCTACAACAACG	TGAGAAGGAATGGATCAGTGG	90
6	CAU-G2-RT06	TGCCATTCAAAAGTTGATAATAAAA	AAAAGGTTGCGACCATGATT	87
7	CAU-G2-RT07	GATTTCTTCTCCTTGCTGCTG	GCAAGAATTAATTGCAGTAAGAGC	99
8	CAU-G2-RT08	GGAGCTCAGACGAGGAACC	TTCCTCTGCTGTGATTAAGTGG	98
9	CAU-G2-RT10	CAGCAAGGACAGAGACATTCC	CTAATCAAGCGGAGGTGGTC	98
10	CAU-G2-RT14	CGACACTTATGACGGGCGCTA	CATGGCAATTGTTAGTGCTCA	89
11	CAU-G2-RT15	GGAGCTCCCCCAAGAGAAG	CCAAGTACATGGGCAGACA	104
12	CAU-G2-RT16	GACTTGGCCAACTACGCATT	AGCTTGTTTCATTGCGTTTTTC	83
13	CAU-G2-RT17	CAAGTCGGAAGGCTATGTCC	CAAGCCGAAAATCCATCAAT	91
14	CAU-G2-RT18	AACTGGAACATGGACCATCC	GTGGAGTGAAACCCAGAGGA	99
15	CAU-G2-RT19	CGGGTGTGAACTTGGAATC	CCGACATGTGAGACTGACTGA	96
16	CAU-G2-RT21	CTTACTCTGTTCCCAGCAGC	GTACCCGAGGACGGAAGC	100
17	CAU-G2-RT22	TGATCAACAAAGTTCAGGGAAG	TGAAGCCGTTCAAGTCAAAA	95
18	CAU-G2-RT23	TTGGTCCTTGGGATCAAGAG	CCGATGAGTGAGGTTTTGCT	94
19	CAU-G2-RT24	TCTTGGACTGAAGCCACCTT	AATATCATCCACACGCTGGAG	92
20	CAU-G2-RT25	CCGGGAGGTTAGAGGAAGAG	TCGACATGGTCAGATCCAAA	87
21	CAU-G2-RT26	TGGTCCTTCAACAAAAGTTGG	TGTTCTAATACAAAATGCCAAAACG	87
22	CAU-G2-RT27	TGCTATAGATGGGACGCAAG	ATTCTCATGGCTGGCAATTT	103
23	CAU-G2-RT28	TTCCGACAGGAAAGCTCAGT	CATCACTGCCTGAACCATTTG	109
24	CAU-G2-RT29	GGAGCCAAGGCTAGGAACAT	TGCTGGTTTTACCACAAAAAT	101
25	CAU-G2-RT30	TTTTTCAGCAAGCTTATTAACACTGA	CGACTTGCTCTAGTCGTTGC	82
26	CAU-G2-RT31	CCATTGATCTTCTTACACAACAATTC	CTCTGGTTGGAGCAAGATGA	100
27	CAU-G2-RT32	TGCTGCTGATACCAGCTTAAT	GAGACCCTCATCACATCCTCA	105
28	CAU-G2-RT33	CTCAGCATCAATGGCAGGT	CTGCTTTTCTCCATCGATCA	109
29	CAU-G2-RT34	CAGACATGAACTCCTGCGTTT	GTTTGGTCGGTTTTGTGGTC	92
30	CAU-G2-RT35	TCCATCTTCCACCAGCTCTC	GCCTTCTGAAAGAACAGGTCA	101
31	CAU-G2-RT36	ATGTCACAGCATTTCGCAGAT	GCCTGAATAAGGCATCGAAC	101
32	CAU-G2-RT37	AAAAAGTTGAAAGAACACTTGGTACA	TGGCAAAATTGTTACTAGTGCAG	98
33	CAU-G2-RT38	CACAACCAGGCAGATCAAGA	TGCCTCTACCTGTTTCAGATCAA	95
34	CAU-G2-RT39	AGCATGCCACACAATATGTCA	TTGTGTGTTGGACAATGTATTCC	97
35	CAU-G2-RT40	CAACGAGGTAATGGTGCAGA	GGACATGGAACATTGTTGGAA	102
36	CAU-G2-RT41	GTGTCCAACCATGAGCTGAG	GCCTCTGTGATTGCCATCTAAT	98
37	CAU-G2-RT42	GTGGAGGGCAAAATTACCAA	ATTCATGCTCCCTCTGTCCA	100
38	CAU-G2-RT43	GAGTACTCCGGCCATGCTTA	CGTACGAACATTTCATGGACA	92
39	CAU-G2-RT44	CAGCTGGAGGAAACAAGAGG	AAGAGAGAGCCCAGTAGCACA	94
40	CAU-G2-RT45	TTGATCAGCTGGAGGAAACA	GCCCAGTAGCACAACCTCTCTG	91
41	CAU-G2-RT46	GGTGACCATCAGACGGTTG	CGAGCCAGACTGCGATGT	90
42	CAU-G2-RT48	TGGGGCAGAGTACTTTGGAT	ACTTGGGGTGTGGGTGTGT	110
43	CAU-G2-RT49	TGTTGATCAGTCCCTCGTCA	TCCATACGATCAGCAATCACA	95
44	CAU-G2-RT50	TTGGTTACCCCAACTTCATGT	GTGGTCAGTGGTCTTGATGG	100
45	CAU-G2-RT51	CGTATCGCTCGGTGTGGT	GAGAGCGAGAGCGAGCAG	103
46	Os-Ubi	GGAGTCACATGCTGCCTAAGGTT	TCACTGCCAGCTTACGGAGG	85

3.9 PCR for identifying differentially expressing *OsGLK* genes

3.9.1 Semi-quantitative PCR targeting *OsGLK*s members

RT primers after standardization on parental genomic DNA were selected for semi quantitative PCR. Semi-quantitative PCR was done on first strand cDNA obtained from control and treated leave samples of two parental genotypes. The first strand cDNAs extracted from control and treated plants were used as template. PCR components used for semi-quantitative PCR were 10-20 ng of cDNA , 1X PCR buffer (Sigma, code: P2192-1VL), 1.2 mM dNTPs (Thermo Scientific, #R0192), 17.5 mM MgCl₂, 1.2 mM primers (F/R), 0.025 U Taq polymerase (Sigma, #D6677-250UN), and made up the volume to 10 µl by adding PCR grade water. PCR condition used was 95 °C initial denaturation followed by 33 cycle PCR consisting of only 10 seconds denaturation step at 95 °C and 30 seconds annealing step at 60°C. The extension step was excluded as the template used was cDNA and the sizes of amplicons were approx 100bp or less. The PCR products were analyzed by electrophoresis using 2 % agarose gel. The gels were visualized in a UV gel documentation system (Alphamager Mini) for analysis of DNA bands and size of the amplified fragments was calculated using 100 bp DNA ladder (Genetix) as size reference standard. The gel pictures were obtained in JPEG format and were used for further analysis. Rice (Ubiquitin) (Yumnam *et al.*, 2015) was used as a housekeeping gene. The band intensity of each GLK members under control and treatment were evaluated using a gel band quantifying software called as GelQuant (Image analysis, processing and quantitation program) (<http://biochemlabsolutions.com/GelQuantNET.html>) and compared with the band intensity of ubiquitin primer (control and treatment separately) to identify the differentially expressed genes. The *OsGLK* family members showing differential gene expression in control and treatment condition were further subjected to real time PCR.

3.9.2 Quantitative/real time PCR

For real time PCR, along with the differentially expressed genes identified in semi quantitative PCR, four *OsGLK* genes identified from root transcriptome data showing differential expression (Tyagi and Rai, 2017) and 10 genes carrying multiple copies of P1BS cis-acting element (PHR1-binding sequence found in the upstream regions of phosphate starvation responsive genes) were used. For real time PCR, along with two parental genotypes (both tolerant to low P), two susceptible genotypes namely, BAM 811 and BAM 350 were also used. PCR components used for quantitative/real time PCR were 10-20 ng of cDNA, 10X real time

PCR master mix (Affymetrix: VeriQuest Fast SYBR Green qPCR master mix, #75690), 1.2 μ M primers (F/R) and made up the volume to 10 μ l by adding PCR grade water. PCR conditions used were similar to semi quantitative PCR. The differential expressions of genes were identified by measuring their Cq values (The cycle in which fluorescence can be detected is termed quantitation cycle (Cq for short)). Similar to semi quantitative PCR, ubiquitin gene specific primer was used as reference to identify the differentially expressing genes. Log₂ fold expression values were calculated for each genes used in real time PCR by using Δ Cq values (Δ Cq= Cq value of a gene - Cq value of ubiquitin (control and treatment separately)) with the formula $2^{(-\Delta\Delta Cq)}$.

3.10 Field Screening of RILs under acidic and low P in low land conditions

The subsequent generations of mapping population/RILs from F₄ to F₆ were grown under lowland acidic field condition. The field data were collected from F₄ generation onwards. Data were collected from total of 1,785 plants representing 5 plants each from 357 lines. The methods and number of plants of each line from which field data were collected is well described in fig 2.1. Data collected on field were as follows;

1. **Tiller number at 30 days after transplanting (TN 30):** Tiller number of plant was recorded after 30 DAT (5 plants from each line).
2. **Tiller number at 60 DAT (TN 60):** Tiller number of plant was recorded after 30 DAT (5 plants from each line).
3. **Days to 50 % flowering (50 % DTF):** Days to 50% flowering data was recorded when 50 % of a particular line in the field flowered (5 plants from each line).
4. **Plant height (cm) (PH):** The height of plant was obtained by measuring from the base of the individual plant to the topmost or tips of the longest panicle with the help of a 1 meter ruler (5 plants from each line).
5. **Panicle length (PN):** The panicle length was measured by taking the length of 3 randomly chosen panicles from each plant with the help of a (30 cm) ruler (5 plants from each line).
6. **Flag leaf area (LA):** The flag leaf length and width was taken from three randomly selected leaves of a plant when the leaves attained their maximum

size. The flag leaf area was calculated by multiplying length and breadth (5 plants from each line).

7. **Panicle number (PN)** Panicle numbers were counted when the plants have reached their maximum growth stage (5 plants from each line).
8. **Plant dry weight at 60 days after transplanting (PDW at 60):** The whole plant was uprooted and they were allowed to dry in green house condition and after drying, dry weight of the whole plant were measured using a weighing balance and were recorded (one representative plant from each line)
9. **Plant dry weight at harvesting (g) (PDW at harvest):** After harvesting, plants were dried in the greenhouse condition. After drying the weight of the plant was measured using weighing balance (5 plants from each line)
10. **Filled Grain (FG):** After harvesting, the total number of filled grains per plant was taken by spreading the seeds on paper and counting seeds using seed counter (Version 1.9.6) mobile application (5 plants from each line)
11. **100 grain weight (100 SW):** The weight of 100 filled grains per plant was taken using an electric weighing balance and were recorded (5 plants from each line).
12. **Spikelet fertility percentage (F %):** It was obtained from the ratio of filled to total number of grains which were expressed as a percentage. (5 plants from each line)

$$F \% = \text{Filled grain} / \text{total number of grain} \times 100$$
13. **Grain yield per plant (g) (GYPP):** Weight of total grains per plant was measured and recorded (5 plants from each line)
14. **P content per mg of dry sample (μg) (P content):** P content in ppm for leave tissue was calculated by the following formula;

$$x \text{ ppm} = x \text{ mg/l}$$
 (a value of 0.11 mg/ g was multiplied to account for dilution factor)
15. **Phosphorus Use Efficiency at 60 days after transplanting (PUE at 60):** PUE at 60 was calculated by dividing dry weight of plant at 60 DAT with leaf P content expressed in g DW/mg Pi.
16. **PUE at harvesting (PUE at harvest):** PUE at harvest was calculated by dividing dry weight of plant at harvest with leaf P content expressed in g DW/mg Pi.

The collected field data were first normalized with the help of data normalization software called as ACBD-R (Augmented Complete Block Design with R) (<https://data.cimmyt.org/dataset.xhtml?persistentId=hdl:11529/10855>). It is a statistical software to simplify the creation and analysis of augmented complete block designs using R.

3.11 Estimation of P from rice plant tissue

3.11.1 Preparation of standard curve

For estimating the P content in plant samples, a standard curve was generated. Procedure for preparing reagents required for standard curve preparation was as follows:

1. Ammonium Molybdate – Ammonium Vanadate solution

With the help of a weighing balance, 22.5 g of ammonium molybdate and 1.25 g of ammonium vanadate were measured and dissolved in 400 ml and 300 ml of distilled water respectively. Ammonium vanadate was dissolved in boiling distilled water. After cooling the ammonium vanadate solution to room temperature, both molybdate and vanadate solutions were mixed together in 1 litre bottle and total volume of 1 litre was made up by adding 250 ml of concentrated HNO_3 (Nitric acid) and rest with distilled water.

2. Phosphate standard solution

With the help of a weighing balance, 21.95 g of analytical grade KH_2PO_4 was measured and dissolved in 1 litre of distilled water making final concentration 50 $\mu\text{g P/ml}$ of solution.

Standard curve was prepared by using different dilution of phosphate standard solution with P concentration ranging from 0 – 30 $\mu\text{g/ml}$. The complete combination and concentration of phosphate standard and vanado-molybdate solution used for preparing standard curve is provided in Table 3.3.

Table 3.3 Combination and concentration of phosphate standard solution, vanado-molybdate solution and distilled water used for preparing standard curve

Phosphate standard sol. (ml)	Vanado-molybdate sol. (ml)	Distilled water (ml)	Final P concentration ($\mu\text{g/ml}$)
0.00	2	2	0.00
0.05	2	1.95	2.50
0.10	2	1.90	5.00
0.15	2	1.85	7.50
0.20	2	1.80	10.00
0.25	2	1.75	12.50
0.30	2	1.70	15.00
0.35	2	1.65	17.50
0.40	2	1.60	20.00
0.45	2	1.55	22.50
0.50	2	1.50	25.00
0.55	2	1.45	27.50
0.60	2	1.40	30.00

Each tube containing different concentration of phosphate standard solution and vanado-molybdate solutions was incubated in dark for about 30 minutes. After 30 minutes of dark incubation, absorbance corresponding to P content from each tube was measured by using spectrophotometer at 420 nm. The graph was prepared by plotting absorbance of each sample against their known concentration.

Phosphorus content in plant sample was determined by vanadomolybdo-phosphoric yellow color method. Dried plant tissue weighing 0.5 gm was taken for digestion. The chopped dry plant sample was digested using 5 ml of di-acid mixture of Nitric acid and Perchloric acid ($\text{HNO}_3 + \text{HClO}_4$) in 3:1 ratio. The mixture was kept in dark condition at room temperature until the mixture turned white. After the samples were digested, it was diluted by adding 50 ml of distilled water. The diluted samples were filtered through Whatman no. 42 in fresh test tubes. A portion of filtrate (1ml) was taken into 25 ml fresh test tubes and to this 2 ml of vanado molybdate reagent was added and then final volume of 10 ml was made up by adding distilled water. The stable yellow color in sample was determined using a spectrophotometer at 420 nm wavelength and concentration of P in each sample was calculated from the

standard curve and the final value of P content in each sample was obtained in terms of $\mu\text{g}/\text{mg}$ of dry plant sample. Phosphorus use efficiency (PUE) was calculated by dividing dry weight of plant with P content expressed in $\text{g DW}/\text{mg Pi}$.

3.12 Genomic DNA isolation from parental genotypes and RILs

Leaf samples were collected from parental genotypes and 357 (F_6) plants representing each line after 30 days of transplanting. Plant genomic DNA was extracted using CTAB (Cetyl-Tri Methyl Ammonium Bromide) extraction method (Murray and Thompson, 1980). The quality and concentration of the DNA was checked in 0.8 % agarose gel by comparing with 1kb DNA ladder. Quantified DNA samples were diluted by using 1X TE (Tris EDTA) buffer to get the final working concentration of 50-70 $\text{ng}/\mu\text{l}$ of gDNA to be used as template for PCR.

3.13 Polymorphism survey in between parental genotypes using molecular markers

After isolating the genomic DNA from two parental genotypes, 416 candidate gene based primers including the primers designed from differentially expressing *OsGLK* family member and *OsGLK* members containing multiple copies of P1BS cis-acting elements, 350 SSRs markers (both genic and non-genic) and 34 SNPs were surveyed for polymorphism between the parental genotypes (Annexure II). PCR components used for polymorphism survey were 1X PCR buffer (Sigma, code: P2192-1VL), 1.2 mM dNTPs, (Thermo Scientific, #R0192), 17.5 mM MgCl_2 , 1.2 μM primers (F/R), 0.05 U Taq polymerase (Sigma, #D6677-250UN), and made up the volume to 10 μl by adding PCR grade water. The PCR condition consist of an initial denaturation of 95°C for 5 minutes followed by 33 cycles of 95°C for 1 minute, 1 minute annealing at 55°C/58°C/60°C and 2 minutes elongation at 72°C. A final extension step for 5 minutes at 72°C was applied. The PCR products were analyzed by electrophoresis using an agarose gel (1.5-3 %). The gels were visualized in a UV gel documentation system (Alphamager Mini) for analysis of DNA bands and size of the amplified fragments was calculated using 100bp/1Kbp DNA ladder as size reference standard. The identified polymorphic markers were used for genotyping of the RILs.

3.14 Selective genotyping of the RILs

After collecting all the phenotypic data, RILs were categorized into two groups viz. better performing lines and bad performing lines on the basis of five yield

attributing traits namely, i) TN 30 ii) TN 60 iii) F % iv) FG and v) 100 SW. From total of 357 RILs, top 40 better performing lines and bottom 40 bad performing lines were selected for selective genotyping of the RILs. The identified parental polymorphic markers were used for genotyping the selected 40 good and bad performing lines respectively. The PCR components and conditions used were similar to the one used in parental polymorphism survey. Allele coming from Sahbhagi Dhan (LR 23) was designated as allele A, allele coming from Chakhao Poreiton (LR 26) was designated as allele B respectively and progeny having alleles from both the parents (heterozygous) was designated as AB.

3.15 Statistical analysis

3.15.1 Correlation and frequency distribution

Data collected in the year 2015, 2016 and 2017 were subjected to correlations analysis. The traits that are strongly correlated with the yield of the plants were identified from each generation. Frequency distribution for each trait on the basis of which good and bad performing lines were selected was calculated from normalized data and plotted in a graph.

3.15.2 Chi square analysis

Chi square test was carried out to test the allelic frequencies observed in the better performing lines and bad performing as against the respective overall frequencies (expected) in the 80 F₆ individuals genotyped. Chi square test using the below formula was performed for goodness of fit to test the observed allelic frequencies as against the expected allelic frequency in tolerant and susceptible bulks.

$$\chi^2 = \sum \frac{(O - E)^2}{E}$$

Where, O= observed frequency in class

E= expected frequency in same class

Σ= summation over all classes

Through Chi square test, markers which were associating with yield attributing traits were identified.

3.15.3 Regression analysis

Using the linear regression model in Microsoft excel, simple regression analysis was conducted for the test of association between markers and traits and to compare and understand the relationship between marker and traits. For regression

analysis the phenotypic characters were considered as the dependent variables, while genotypic values or the marker genotype were considered as independent.

3.16 Population structure analysis

Percentage of parental ancestry for the genotypes used for selective genotyping was determined by using Bayesian cluster estimation programme STRUCTURE Version 2.3.2.1 (Pritchard *et al.*, 2000). Three number of clusters (K= 2 to 4) were considered with ten replicates for each value of K. Each run used a burn-in period of 100,000 iterations followed by 50,000 iterations. The best replicate which give the maximum likelihood at K = 2 (since samples were a bi-parental population) were chosen for the final result analysis. The range of optimal K (number of populations) values to be tested was determined based on genotypes used and marginal likelihood to explain the distribution of data. Net nucleotide distance between the clusters and fixation index (F_{ST} value) as a measure of population differentiation due to genetic structure was also calculated (Wright's statistics). The classification of each accession (LR 23 type or LR 26 type) was based on its inferred ancestry. If the maximum value was less than 80 %, it was classified as admix.

3.17 Genotyping whole populations of RILs

Markers which were found to be significantly associating with specific yield attributing traits were further used for genotyping all 357 RILs. The PCR components and conditions used were similar to the one used in parental polymorphism survey. The marker data were collected as allele A (LR 23 allele), B (LR 26 allele), AB (Heterozygous) or absent. Using the linear regression model in Microsoft excel, simple regression analysis was conducted for the test of association between markers and traits and to compare and understand the relationship between marker and traits in whole population.

Chapter 4

Results

The aim of the current study was to study in detail one of the rice transcription factor family, GLK and to understand tolerance to low P under acidic soil conditions using a biparental population. The results obtained are detailed below.

4.1 Basic information of members of OsGLK family

The basic information regarding *OsGLK* family was obtained using various online databases mentioned in Materials and Method chapter. MYB transcription factor family, *OsGLK*, belongs to super family GARP and has 62 members in *Oryza sativa japonica* type and 52 members in *indica* type. Out of a total of 62 *OsGLK* genes available in the databases, 45 codes for a functional gene and the rest 17 are splice variants. For two of the members (*Os03g45194* and *Os01g13740*), a name already exists in the online databases, viz., *OsGLK1* and *OsGLK2*, respectively. For rest of the members new names were assigned in sequential order following the name pattern already available in the database (Table 4.1).

For the ease of writing, from this point forward, *OsGLK* members have been simply written as K.

Using Map tool available in Oryzabase database, the chromosomal map of all K members were generated, the map chromosomal map position showed that the genes are present on all the 12 chromosomes of rice with maximum number of K genes in chromosome 2 (*K6*, *K7*, *K8*, *K18*, *K48*, *K49* and *K50*). Six genes were found each on chromosome 1 (*K2*, *K3*, *K4*, *K5*, *K26* and *K52*), chromosome 3 (*K14*, *K15*, *K16*, *K17*, *K18* and *K51*) and chromosome 6 (*K1*, *K25*, *K28*, *K29*, *K30* and *K31*); five genes on chromosome 8 (*K34*, *K35*, *K36*, *K37* and *K38*); four genes on chromosome 5 (*K21*, *K22*, *K23*, and *K27*), three genes on chromosome 9 (*K39*, *K40* and *K41*), two genes each on chromosome 7 (*K32* and *K33*), chromosome 10 (*K42* and *K43*) and chromosome 12 (*K45* and *K46*) and one gene each on chromosome 4 (*K19*) and chromosome 11 (*K44*) (Fig. 4.1).

Table 4.1 List of OsGLK family members with their locus id, original name available and new name assigned

Locus id	Original name available	New name assigned	Locus id	Original name available	New name assigned
LOC_Os06g24070	<i>OsGLK1</i>	<i>OsGLK1</i>	LOC_Os06g45890	N/A	<i>OsGLK30</i>
LOC_Os01g13740	<i>OsGLK2</i>	<i>OsGLK2</i>	LOC_Os06g49040	N/A	<i>OsGLK31</i>
LOC_Os01g62660	N/A	<i>OsGLK3</i>	LOC_Os07g25710	N/A	<i>OsGLK32</i>
LOC_Os01g67770	N/A	<i>OsGLK4</i>	LOC_Os07g48596	N/A	<i>OsGLK33</i>
LOC_Os01g74020	N/A	<i>OsGLK5</i>	LOC_Os08g06370	N/A	<i>OsGLK34</i>
LOC_Os02g04640	N/A	<i>OsGLK6</i>	LOC_Os08g25799	N/A	<i>OsGLK35</i>
LOC_Os02g07170	N/A	<i>OsGLK7</i>	LOC_Os08g25820	N/A	<i>OsGLK36</i>
LOC_Os02g07770	N/A	<i>OsGLK8</i>	LOC_Os08g33050	N/A	<i>OsGLK37</i>
LOC_Os02g22020	N/A	<i>OsGLK10</i>	LOC_Os08g33750	N/A	<i>OsGLK38</i>
LOC_Os03g20900	N/A	<i>OsGLK14</i>	LOC_Os09g12750	N/A	<i>OsGLK39</i>
LOC_Os03g21240	N/A	<i>OsGLK15</i>	LOC_Os09g12770	N/A	<i>OsGLK40</i>
LOC_Os03g45194	N/A	<i>OsGLK16</i>	LOC_Os09g23200	N/A	<i>OsGLK41</i>
LOC_Os03g55590	N/A	<i>OsGLK17</i>	LOC_Os10g32600	N/A	<i>OsGLK42</i>
LOC_Os03g55760	N/A	<i>OsGLK18</i>	LOC_Os10g39550	N/A	<i>OsGLK43</i>
LOC_Os04g47890	N/A	<i>OsGLK19</i>	LOC_Os11g01480	N/A	<i>OsGLK44</i>
LOC_Os05g34110	N/A	<i>OsGLK21</i>	LOC_Os12g01490	N/A	<i>OsGLK45</i>
LOC_Os05g40960	N/A	<i>OsGLK22</i>	LOC_Os12g39640	N/A	<i>OsGLK46</i>
LOC_Os05g41240	N/A	<i>OsGLK23</i>	LOC_Os02g14490	N/A	<i>OsGLK48</i>
LOC_Os06g35140	N/A	<i>OsGLK25</i>	LOC_Os02g45080	N/A	<i>OsGLK49</i>
LOC_Os01g20700	N/A	<i>OsGLK26</i>	LOC_Os02g46940	N/A	<i>OsGLK50</i>
LOC_Os05g32880	N/A	<i>OsGLK27</i>	LOC_Os03g03760	N/A	<i>OsGLK51</i>
LOC_Os06g40710	N/A	<i>OsGLK28</i>	LOC_Os01g08160	N/A	<i>OsGLK52</i>
LOC_Os06g45410	N/A	<i>OsGLK29</i>			

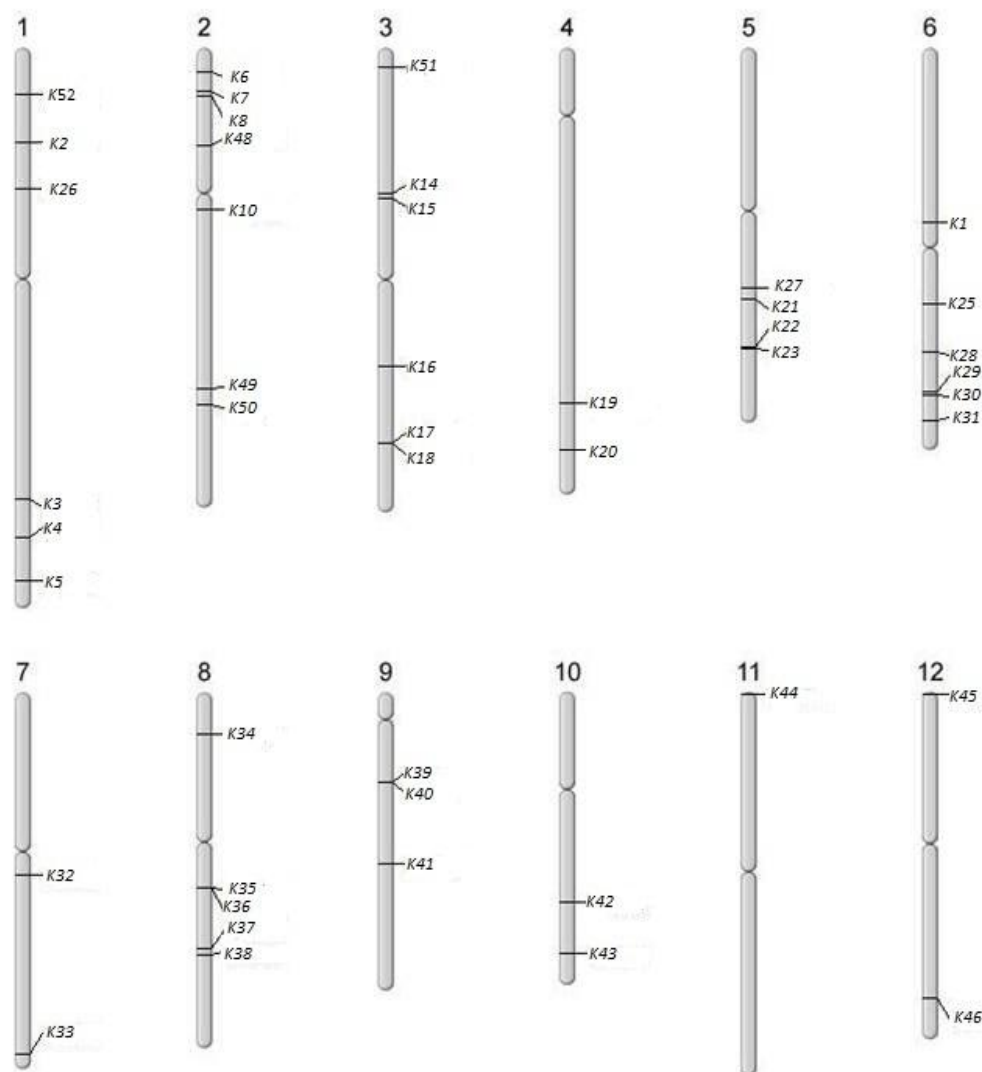


Fig. 4.1 Chromosomal map showing distribution of *OsGLK* genes on 12 rice chromosomes

4.1.1 Sequence information

The average length of coding DNA sequence (CDS) and amino acid (aa) sequence of *K* was found to be 1229 bases and 372 amino acid, respectively. The longest CDS and amino acid length of 1749 and 583 aa was found in *K4* and the shortest CDS and amino acid length of 717 bases and 239 aa in *K5*.

4.1.2 Gene orientation

For all the members of *OsGLK*, the information regarding orientation of the gene in the genome are obtained from online database *i.e.* RiceGE: Rice Functional Genomic Express Database. It was found that 25 members are in upward orientation and 20 are in downward orientation (Table 4.2).

4.1.3 Expression in different tissues

The expression of members of *OsGLK* was found in different rice tissues like leaf, flower, panicle, seed, stem, seed, root, callus and vegetative meristem, etc. The information regarding tissue-level expression was not available in the online databases for nine members namely, *K5*, *K26*, *K36*, *K42*, *K43*, *K45*, *K48*, *K49* and *K50* (Table 4.2)

4.1.4 Orthologs in maize

Orthologs of the members of *OsGLK* genes are available in the online database, *i.e.* Rice Genome Annotation Project Database and Plant Transcription Factor Database. Using these two database, it is found that maize ortholog of almost all the members of *K* gene are available except for *K7*, *K26* and *K27*. The maize ortholog id available for each *K* members is given in Table 4.2.and 4.3.

Table 4.2 Basic information of thirty three *OsGLK* genes and their maize orthologs

Name	CDS length	Amino acid length (aa)	Gene orientation	Expressed in	Ortholog in maize
K1	1368	456	Upward	Callus, Leaf, Panicle, Root, Stem	GRMZM2G026833
K2	1620	540	Downward	Flower, Leaf, Panicle, Seed, Stem	GRMZM2G087804
K3	762	254	Upward	Callus, Flower, Stem	GRMZM2G074908
K4	1749	583	Downward	Leaf, Seed, Stem	GRMZM2G177220
K5	717	239	Downward	N/A	GRMZM2G067702
K6	1404	468	Downward	Callus, Flower, Leaf, Root, Stem	GRMZM2G010920
K7	708	236	Downward	Panicle	N/A
K8	1068	356	Downward	Stem	GRMZM2G052544
K10	1239	413	Upward	Callus, Leaf	GRMZM2G016370
K14	1065	355	Upward	Leaf, Panicle, Stem	GRMZM2G009060
K15	1287	429	Upward	Callus, Flower, Leaf, Panicle, Root	GRMZM2G006477
K16	1671	557	Downward	Panicle	GRMZM2G065194
K17	1047	349	Upward	Callus, Flower, Leaf, Panicle, Stem	GRMZM2G124540
K18	1179	393	Upward	Panicle	GRMZM2G031870, GRMZM6G039492
K19	921	307	Upward	Callus, Panicle	GRMZM2G069525, GRMZM2G090230
K21	996	332	Downward	Flower, Panicle	GRMZM2G398055, GRMZM2G471600
K22		412	Downward	Leaf	GRMZM2G701218
K23	774	258	Downward	Callus, Flower, Leaf, Panicle, Seed, Stem	GRMZM2G117854
K25	1632	544	Downward	Callus, Root	GRMZM2G333083
K26	1032	344	Upward	N/A	N/A
K27	1542	514	Downward	Flower	N/A
K28	990	330	Upward	Callus, Flower, Panicle, Vegetative meristem	GRMZM2G173943
K29	1098	366	Upward	Callus, Leaf, Panicle	GRMZM2G081671
K30	771	257	Upward	Callus, Flower, Leaf, Panicle, Seed, Stem	GRMZM2G454449
K31	1440	480	Upward	Flower, Leaf, Panicle, Seed, Stem	GRMZM2G083472
K32	1281	427	Upward	Callus, Flower, Leaf, Panicle, Root, Seed, Stem	GRMZM2G162409
K33	1038	346	Downward	Leaf, Seed, Stem	GRMZM2G009060
K34	969	323	Upward	Panicle	GRMZM2G123308,
K35	741	247	Upward	Callus, Flower, Leaf, Panicle, Seed, Stem	GRMZM2G113742
K36	924	308	Upward		GRMZM2G173943
K37	1395	465	Upward	Callus	GRMZM2G056400
K38	891	297	Downward	Callus, Flower, Panicle, Root, Seed, Stem	GRMZM2G117193
K39	753	251	Upward	Callus, Flower, Leaf, Panicle, Seed, Stem,	GRMZM2G064197

Table 4.3 Basic information of twelve *OsGLK* genes and their maize orthologs

K40	867	289	Upward	Callus, Flower, Leaf, Panicle, Root, Stem	GRMZM2G173943
K41	1599	533	Upward	Roots, Stems, Leaves, Flowers	GRMZM2G082264
K42	504	168	Downward	N/A	GRMZM2G379656
K43	990	330	Downward	N/A	GRMZM2G374986, GRMZM5G887276
K44	966	322	Downward	Callus, Leaf, Panicle	GRMZM5G846506
K45	945	315	Downward	N/A	Do
K46	1188	396	Upward	Leaf	GRMZM2G171468
K48	1638	546	Upward	N/A	GRMZM2G477238
K49	762	254	Upward	N/A	GRMZM2G106185
K50	945	315	Upward	N/A	GRMZM2G060834,
K51	1191	397	Downward	Flower, Panicle	GRMZM2G374986,
K52	1524	508	Upward	Leaf, Panicle, Root	GRMZM2G348238

4.1.5 Amino acid frequency

Amino acid frequency in each member was calculated with the help of sequence alignment tool available in MEGA 5.2 software. The detail information regarding frequency of amino acid present in each member is given in Annexure. Based on the average amino acid frequency present *OsGLK* members, the highest frequency was obtained for amino acid serine (10.71 %) and the lowest for amino acid tryptophan (0.93%) (Table 4.4).

Table 4.4 Average amino acid frequency present in *OsGLK* members

Amino acid	(%)	Amino acid	(%)	Amino acid	(%)	Amino acid	(%)
Alanine	9.6	Glycine	9	Methionine	3	Serine	10.71
Cystine	1.3	Histidine	4	Asparagine	3	Threonine	4.9
Aspartic acid	5.3	Isoleucine	3	Proline	7	Valine	5
Glutamic acid	5.9	Lysine	5	Glutamine	5	Tryptophan	0.93
Phenylalanine	2.3	Leucine	8	Arginine	6	Tyrosine	1.7

4.1.6 Molecular and biological function

As the gene belongs to transcription factor family, the molecular function of all the members are sequence specific DNA binding. However, different biological functions such transcription factor for phosphate starvation response, for chloroplast development, PSII associated light-harvesting complex II catabolic process, chlorophyll catabolic process, oxidation-reduction process, flower development, multicellular organismal development, response regulator involved in His-to-Asp phosphorelay signal transduction system, phloem and xylem development and transcription factor for the generation of the circadian clock oscillation (Table 4.5)

were identified. For K26, the information about its biological function is not available in the database.

Table 4.5 Annotated biological function of the *OsGLK* genes

Name	Biological function	Name	Biological function
K1	Chloroplast development	K30	Regulation of transcription (OsMPH1)
K2	Chloroplast development (similar to <i>Psr1</i>)	K31	Regulation of transcription (PHR4) (Ruan <i>et al.</i> , 2017)
K3	Nucleic acid metabolic process (Homeodomain like protein)	K32	Transcription factor involved in phosphate starvation signaling.
K4	(B type response regulator 6; RR6)	K33	Regulation of transcription
K5	Essential for circadian clock oscillation	K34	Regulation of transcription
K6	Involved in phosphate starvation signaling	K35	Regulation of transcription
K7	Regulation of transcription	K36	Regulation of transcription
K8	Xylem and Phloem development	K37	Nucleic acid metabolic process, flower development, cell differentiation, anatomical structure morphogenesis
K10	Regulation of transcription (<i>NIGT1/SPX2</i>) Nitrate induced GARP type transcriptional repressor 1 (Kiba <i>et al.</i> , 2018)	K38	Regulation of transcription
K14	Regulation of transcription, similar to <i>PSR1</i>	K39	Cellular response to phosphate starvation
K15	Transcription factor involved in phosphate starvation signaling (<i>PHR1</i>) (Zhou <i>et al.</i> , 2008)	K40	Regulation of transcription (OsMYBc; regulation of high affinity K transport; salt tolerance (Wang <i>et al.</i> , 2015)
K16	PSII associated light-harvesting complex II catabolic process	K41	Inflorescence development, plant ovule development (RL9,SL1), polarity specification of adaxial/abaxial axis, radial pattern formation, xylem and phloem pattern formation
K17	Regulation of transcription (Homeodomain like protein)	K42	Functions as a response regulator involved in His-to-Asp phosphorelay signal transduction system
K18	Regulation of transcription, integument development	K43	Regulation of transcription
K19	Regulation of transcription (Homeodomain like protein)	K44	Do
K21	Regulation of transcription	K45	Do
K22	Regulation of transcription, similar to <i>Psr1</i>	K46	Do
K23	Regulation of transcription	K48	Do
K25	Regulation of transcription	K49	Do
K26	N/A	K50	Flower development
K27	B type response regulator 6; RR6)	K51	Regulation of transcription, <i>OsMPH1</i> homolog (Zhang <i>et al.</i> , 2017)
K28	Regulation of transcription	K52	Response to temperature stimulus, gibberellic acid mediated signaling pathway, histone H3-K36 methylation, negative regulation of long-day photoperiodism, flowering
K29	Do		

4.1.7 Protein annotation

On the basis of protein annotation data available on online databases, OsGLK proteins were categorized into three major classes a) two component response regulator (K4, K7, K10, K27 and K42), b) phosphate starvation response regulator (K6, K14, K15, K23, K31, K32, K36 and K40) and c) transcription factor (K2, K5, K8, K18, K19, K21, K1, K29, K30, K33, K34, K35, K37, K38, K39, K44, K45, K50 and K51) and rest of the proteins are still not characterized (Table 4.6).

Table 4.6 Details of protein annotation for members of the *OsGLK* family

Name	Protein annotation	Name	Protein annotation
K1	Probable transcription factor GLK1	K30	Probable transcription factor KAN3
K2	Probable transcription factor GLK2 isoform X1	K31	Protein PHR1-LIKE 1
K3	WAS protein family homolog 1 isoform X1	K32	Protein PHR1-LIKE 1
K4	Two-component response regulator ORR26	K33	Myb family transcription factor APL isoform X2
K5	Transcription factor PCL1	K34	Probable transcription factor KAN2 isoform X2
K6	Protein PHR1-LIKE 1	K35	Myb family transcription factor APL
K7	Two-component response regulator ARR18	K36	Protein PHR1-LIKE 1
K8	Myb family transcription factor APL isoform X1	K37	Probable transcription facto RL9
K10	Two-component response regulator ORR22	K38	Myb family transcription factor APL
K14	Protein PHR1-LIKE 1 isoform X1	K39	Myb family transcription factor APL
K15	Protein PHR1-LIKE 1	K40	Protein PHR1-LIKE 1 isoform X1
K16	Hypothetical protein SORBIDRAFT_01g013080	K41	Probable transcription factor RL9
K17	uncharacterized protein	K42	Two-component response regulator ORR30 isoform X1
K18	Transcription factor LUX	K43	Uncharacterized protein
K19	Putative Myb family transcription factor	K44	Probable transcription factor RL9
K21	Transcription factor PCL1-like	K45	Probable transcription factor RL9
K22	Uncharacterized protein	K46	Uncharacterized protein
K23	Protein PHR1-LIKE 1	K48	Uncharacterized protein
K25	Uncharacterized protein	K49	Uncharacterized protein
K26	Uncharacterized protein	K50	Probable transcription factor KAN2 isoform X2
K27	Two-component response regulator ORR27	K51	Probable transcription factor RL9
K28	Uncharacterized protein	K52	Uncharacterized protein
K29	Myb family transcription factor APL		

4.2 Gene disruption phenotype

Gene disruption phenotype for three members (*K4*, *K27*, and *K41*) is available in the databases. Phenotype of disruption in *K4* is dwarf, narrow leaf, low tillering, late heading and low fertility. The mutant phenotype in *K27* is dwarf with narrow leaf, mimic lesion, low tillering having altered kernel color, viviparous and low fertility. The mutant phenotype for *K41* shows rolled inward leaves like a cylinder at mature stage, malformed spikelets with low seed sets, light delay in heading date, disordered and irregular arrangement of chloroplast grana lamellae, respectively. The mutant lines are also available for all the members except for *K5*, *K8*, *K14* and *K30* (Table 4.7 and 4.8).

Table 4.7 Available mutant id of twenty *K* members

Gene name	Mutant id	Gene name	Mutant id
<i>K1</i>	FN_N1-202-3, PFG_1D-2707535, AF318581.1,	<i>K30</i>	N/A
<i>K2</i>	M0097359_1, PFG_1C-10532, FN1554-S, CW-631-3-1, PFG_3A-13668	<i>K31</i>	FN1504-S, M0106215_1, M0118169_1
<i>K3</i>	PFG_3B-00518, PFG_1A-10705, M0112820_1, M0112820_1, FN1112-S, FN520-S	<i>K32</i>	FN1324-S, RMD_TTL-04Z11NL88_NTLB5-1, RMD_04Z11NL88
<i>K4</i>	PFG_1A-10902, PFG_2A-50093, PFG_3A-08003, PFG_1C-05813, RGT6114A_5.1,	<i>K33</i>	T34129T, M0079223_1, RMD_03Z11KG31-3, FL025685, FL069991, FL064770, FL064762, FL061791, PFG_3D-00749, RMD_03Z11CT46-2,
<i>K5</i>	N/A	<i>K34</i>	PFG_1D-05826, M0087202_2, FN1001-S, RMD_TTL-03Z11BW18_NTLB5,
<i>K6</i>	NF3709_0_101_1A, NC2707_0_404_1A, T13924T, T38437T, T13664T, NE4507_0_706_1A,	<i>K35</i>	RMD_03Z11KH78, RMD_TTL-03Z11KH78_NTLB5
<i>K7</i>	PFG_3A-60095, PFG_3A-05535, M0074767_1, RMD_03Z11EK12, M0061159_1	<i>K36</i>	PFG_4A-03667, PFG_4A-03762
<i>K8</i>	N/A	<i>K37</i>	M0108166_1, M0108170_1, PFG_3A-03289, SHIP_ZSF6040, PFG_3A-07524, RdSpm4369B_3.1, FN1031-S,
<i>K10</i>	M0099964_1, M0026373_1, PFG_3A-60284, PFG_5A-00381, RdSpm4311B_3	<i>K38</i>	M0118324_1, RMD_TTL-04Z11EX56_NTLB5, H0533_2_102_1A, M0099494_1
<i>K14</i>	N/A	<i>K39</i>	PFG_3A-50632, RMD_04Z11JO31, PFG_1D-03123, RMD_TTL-04Z11JO31_NTLB5, M0101789_1

Table 4.8 Available mutant id of twenty-five K members

K15	PFG_2A-40085, FN968-S	K40	FN469-S, PFG_K-00126,
K16	FN-157-S, FN836-S, PFG_3A-09440, FL017161	K41	FN987-S, RdSpm3963A_3.1, PFG_K-01898, FJ268747.1, SHIP_ZS2416.C, M0095090_1
K17	RGT2334_5.1, RGT3304_5.1, PFG_1B-20821, PFG_K-01744, PFG_3A-11085	K42	RMD_04Z11BE37, RMD_ITR-03Z11CH94_URB4, RMD_03Z11CH94-3
K18	SAK3H11, PFG_4A-50458, PFG_4A-01143, RMD_TTL-03Z11FH88_NTLB5-2, RMD_03Z11FH88-2, RdSpm5010B_3.1	K43	T26355T, T26442T, FL023164, NF9866_0_701_1A, FN757-S, NF9842_0_701_1A, T11671T, NF9872_0_702_1A, T34034T PFG_1B-18707, PFG_3C-00176
K19	M0026507_1, RdSpm2736C_3.1, PFG_2D-01337.L	K44	GU046789.1, FL027850, RMD_04Z11BY51-2, RMD_TTosL-04Z11BY51_TosRS, RdSpm5077D_3.1, RdSpm5077C_3.1, RdSpm5077_3.1, RMD_04Z11QZ45, RMD_TTL-04Z11QZ45_NTLB5, RdSpm5079A_3.1
K21	RMD_TTL-04Z11CS51_NTLB5, FN-59.S, RMD_04Z11CS51, RMD_04Z11OF33, RMD_04Z11CS72, M0046259_1	K45	M0004272_2, 06ET3_387_D_003_017_017A01, T25245T, NF8528_0_701_1A, NF6031_0_404_1A
K22	PFG_1B-14938	K46	RdSpm908_3.1, PFG_2A-50190
K23	FN1188-S, FN-119-S, RMD_04Z11DI38, RMD_TTL-04Z11DI38_NTLB5	K48	M0012990_1, FN735-S, RGT2049_5.1, PFG_1C-05009, FN_769-4-1
K25	FN-187, FN761-S, FN-274, FN1084-S, M0012330_1	K49	RMD_ATosR-04Z11AX46_TosRS, FN1024-S, RdSpm1910B_3.1, PFG_3A-18186, FL045776, M0016664_1,
K26	RdSpm3898A_3.1	K50	CRS211_TT65-61-1-39_Ds3, PFG_1D-2707137
K27	SHIP_NE2037, PFG_1D-03255, PFG_1D-05651	K51	PFG_3A-11060, M0052427_1, RMD_ATosR-05NPBMW35_TosRS, RMD_ATosR-05NPBNV84_TosRS, PFG_3A-03255, PFG_3A-08082, M0112569_1, M0123785_1, PFG_1A-07822, FN1228-S, RdSpm1960B_3.1, M0123785_2
K28	FN630-S, NF3001_0_435_1A, NF3001_0_534_1A, NF3010_0_131_1A, NF3020_0_101_1A, T36636T	K52	RdSpm1683_3.1,
K29	FN1237-S, PFG_3D-01870		

4.3 *Cis*-acting elements in 5' UTR of *OsGLK* members

Information regarding *cis*-acting elements present in 1 Kbp upstream of CDS (5'UTR) is helpful in understanding the molecular function of transcription factors in greater detail. Important *cis*-acting elements with respect to stress or environmental response like GT1 CONSENSUS, MYB1AT, ERELEE4, GCCCORE, P1BS, LTRECOREATCOR15, IBOXCORE, CBFHV, GT1GMSCAM4, MYC CONSENSUSAT were identified (Table 4.9). The types of *cis*-acting elements and their position in the 5' UTR is given in Annexure 1.

Table 4.9 Function of major *cis*-acting elements present in 5' UTR of *OsGLKs*

Cis element site id	Functions	References
TATABOX	Core promoter element	Shirsat <i>et al.</i> , 1989; Grace <i>et al.</i> , 2004
CAATBOX	Common sequence found in the 5'-non-coding regions of eukaryotic genes	Shirsat <i>et al.</i> , 1989
GATABOX	Required for high level, light regulated, and tissue specific expression	Lam and Chua, 1989; Reyes <i>et al.</i> , 2004
GT1 CONSENSUS	Consensus GT-1 binding site in many light-regulated genes	Villain <i>et al.</i> , 1996; Zhou, 1999
MYB1AT	MYB recognition site found in the promoters of the dehydration-responsive gene	Abe <i>et al.</i> , 2003
ERELEE4	ERE (ethylene responsive element) involved in the senescence-related expression of the carnation glutathione-S-transferase (GSTI) gene.	Montgomery <i>et al.</i> , 1993; Rawat <i>et al.</i> , 2005
MYC CONSENSUSAT	Binding site of ICE1 (inducer of CBF expression 1) that regulates the transcription of CBF/DREB1 genes in the cold in Arabidopsis	Chinnusamy <i>et al.</i> , 2003
GT1GMSCAM4	GT-1 motif" Plays a role in pathogen- and salt-induced SCaM-4 gene expression	Park <i>et al.</i> , 2004
CBFHV	Binding site of barley (H.v.) CBF binding factors; CBFs are also known as dehydration-responsive element (DRE) binding proteins (DREBs)	Xue, 2002
GCCCORE	Core of GCC-box found in many pathogen-responsive genes, has been shown to function as ethylene-responsive element,	Brown <i>et al.</i> , 2003; Chakravarthy <i>et al.</i> , 2003
P1BS	PHR1-binding sequence found in the upstream regions of phosphate starvation responsive genes	Rubio <i>et al.</i> , 2001; Schünmann <i>et al.</i> , 2004
IBOXCORE	Conserved sequence upstream of light-regulated genes	Martínez-Hernández <i>et al.</i> , 2002
INRNTPSADB	Inr (initiator)" elements found in the tobacco psaDb gene promoter without TATA boxes	Nakamura <i>et al.</i> , 2002
LTRECOREATCOR15	Cis element for cold-, drought and ABA regulated gene expression	Baker <i>et al.</i> , 1994; Jiang <i>et al.</i> , 1996

Seventeen members of *OsGLK* carry copies of P1BS cis-acting elements (Table 4.10). P1BS is a PHR1-binding sequence found in the upstream regions of phosphate starvation responsive genes identified from several plant species.

Table 4.10 List of *OsGLK* genes carrying multiple copies of P1BS element

Gene name	P1BS		Gene name	P1BS		Gene name	P1BS
K2	2 copies at 775(±)		K17	2 copies at 550(±)		K40	2 copies at 252(±)
K3	2 copies at 652(±)		K22	2 copies at 519(±)		K41	2 copies at 492(±)
K4	2 copies at 288(±)		K25	2 copies at 145(±)		K44	2 copies at 29(±)
K7	2 copies at 618(±)		K30	2 copies at 313(±)		K45	2 copies at 52(±)
K10	4 copies at 241(±), 671(±)		K31	4 copies at 304(±), 342(±)		K51	2 copies at 552(±)
K15	2 copies at 159(±)		K34	2 copies at 736(±)			

4.4 Phylogenic analysis using protein sequence

Phylogenic analysis showed the clustering of *OsGLK* members into many sub-clusters (Fig. 4.2). By using Multiple EM for Motif Elicitation (MEME) software for motif alignment of two major domains a) MYB homeodomain-like and b) MYB CC-type, it was found that position and types of amino acids in these two motifs are conserved across all members (Fig. 4.3).

When protein domain structure and position was aligned with each of the respective members, it was found that the clustering was on the basis of presence and combination of 5 major protein domains, namely a) coil type, b) MYB homeodomain-like, c) signal transduction response regulator domain, d) MYB CC type transcription factor domain, and e) short chain dehydrogenase/reductase domain (Fig. 4.4).

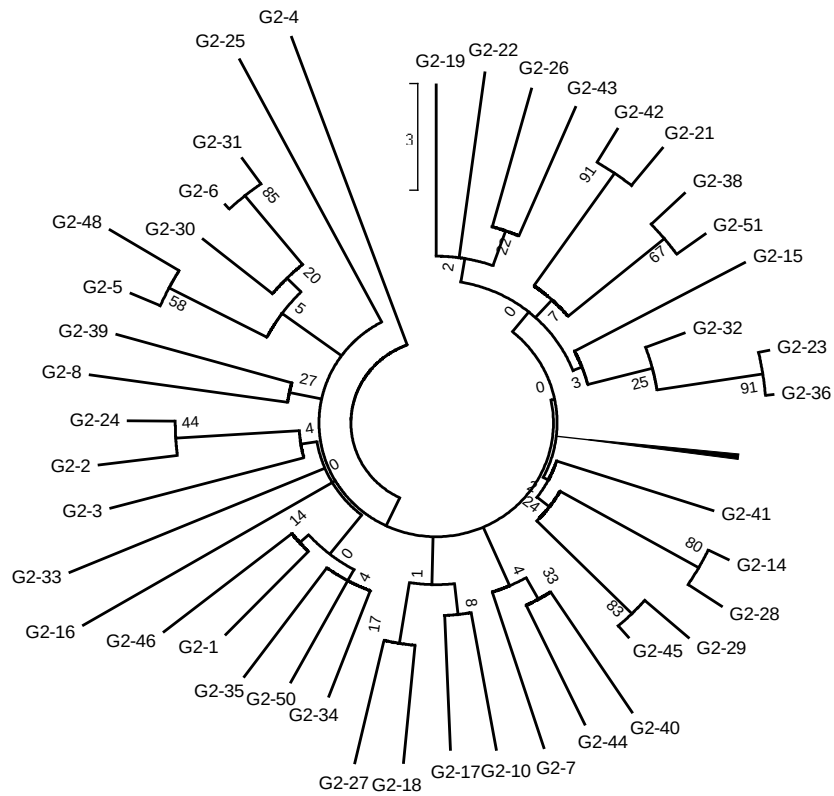


Fig. 4.2 Phylogenetic tree of *OsGLK* members based on amino acid sequence generated by using neighbour joining method calculated with MEGA 5.2 software using 1000 bootstrapping. Bar: 0.3 substitution per amino acid position

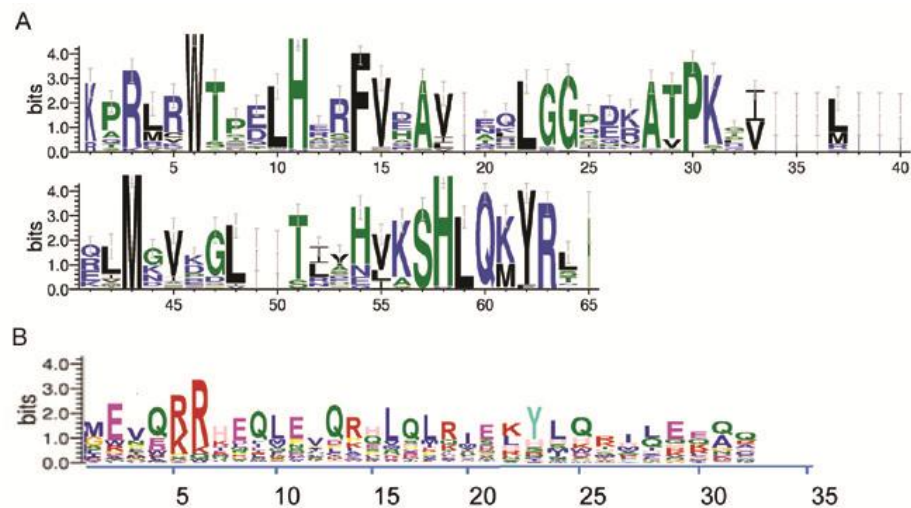


Fig. 4.3 The conserved domains in the *OsGLK* proteins. Logos of the protein alignment of (A) MYB/homeodomain-like and (B) MYB-CC type transcription factor. The visual representation of the identified common motifs is shown. Height of the individual letters in a stack is the probability of the letter at that position multiplied by the total information content of the stack. X-axis shows the position of the amino acid

4.5. Semi quantitative PCR outcome

In order to characterize OsGLK members further, the role of various members in response to low P after 24 hours was checked. Forty five RT primers (Table no. 3.2) were first standardized on genomic DNA. Subsequently, 31 RT primers that showed single sharp band for semi quantitative PCR were selected for further analysis on cDNA. The semi RT PCR was performed as detailed in Materials and Method section. After comparing the band intensity of each amplicon using Gel-quant software and subsequent analysis by comparing the differences in band intensity between the control and 24 hours treated sample, it was found that *K14* was significantly up-regulated in LR 23 and *K37* is significantly down-regulated in LR 26, respectively (Fig. 4. 5, Fig. 4.6 and Fig. 4.7).

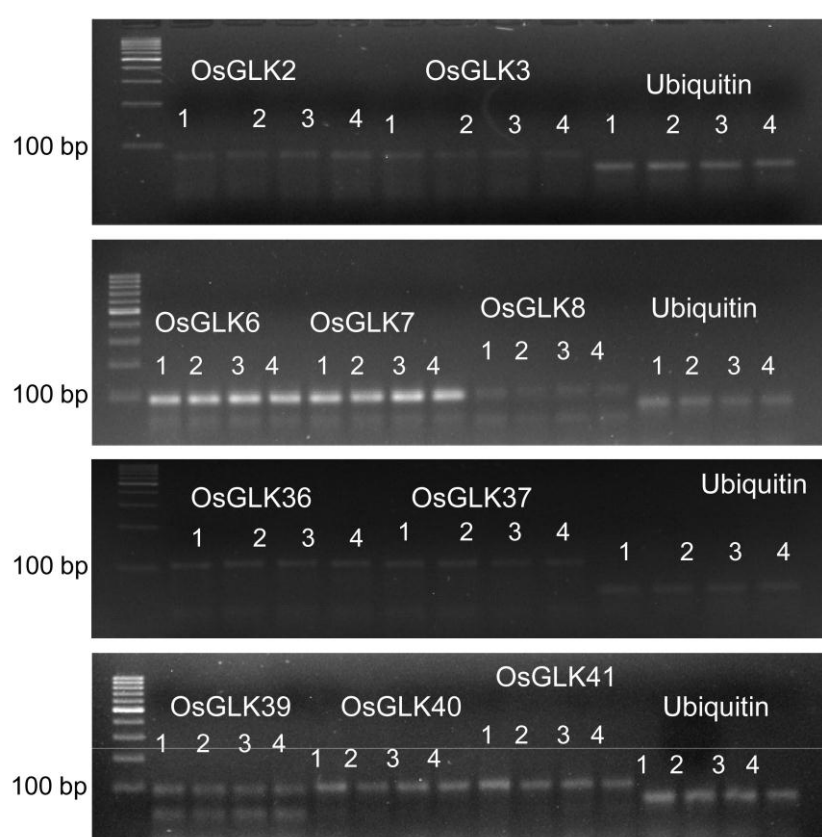


Fig. 4.5 Agarose (2 %) gel picture of semi-quantitative PCR for four OsGLK members. The names of the genes are indicated at the top of the band. Ubiquitin was used for normalisation of data. Samples 1, 2, 3 and 4 indicate LR 23 control (normal P level), LR 23 stress (low P level), LR 26 control (normal P level), LR 26 stress (low P level), respectively.

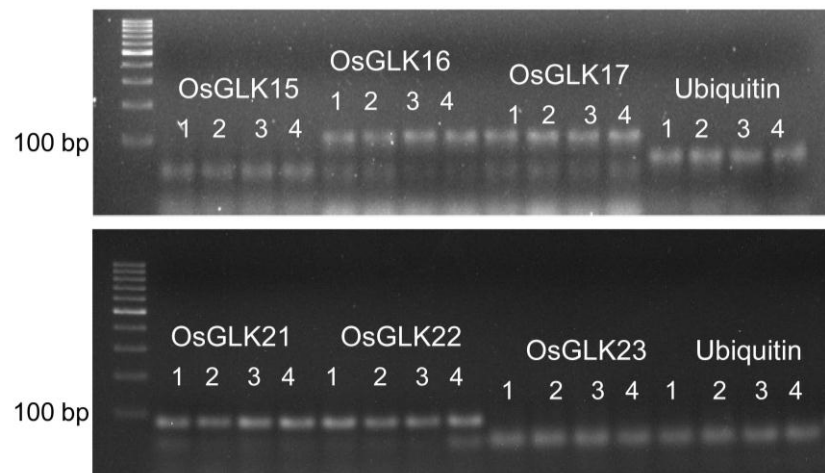


Fig. 4.6 Agarose (2 %) gel picture of semi-quantitative PCR for two OsGLK members. The names of the genes are indicated at the top of the band. Ubiquitin was used for normalisation of data. Samples 1, 2, 3 and 4 indicate LR 23 control (normal P level), LR 23 stress (low P level), LR 26 control (normal P level), LR 26 stress (low P level), respectively.

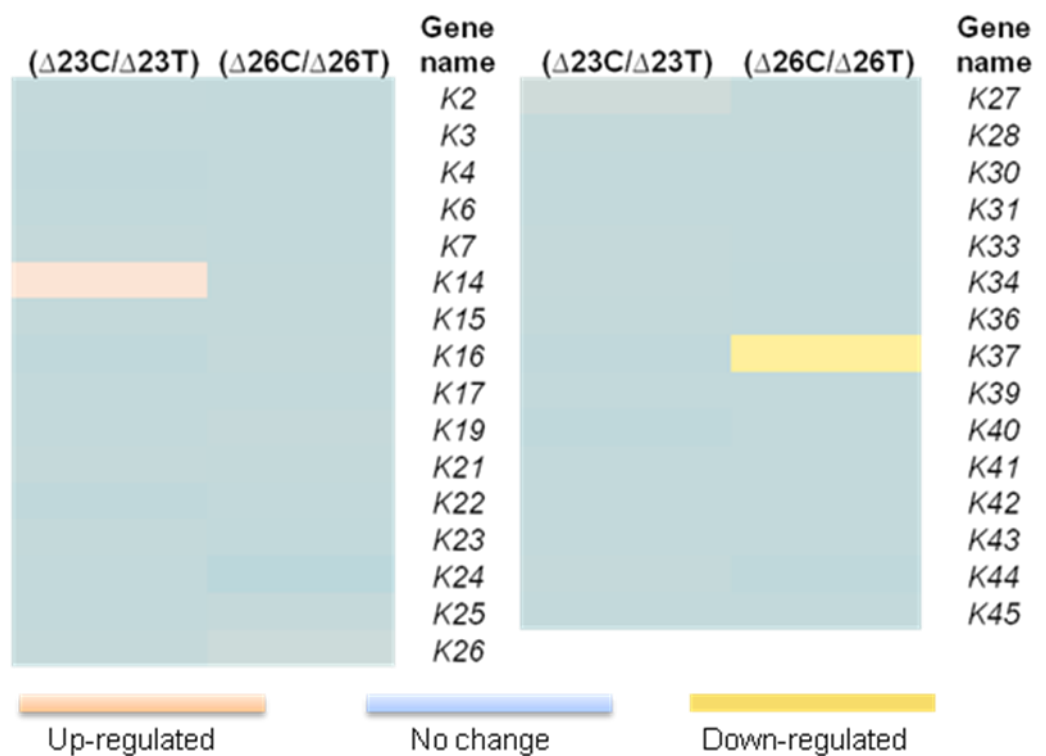


Fig. 4.7 Semi quantitative PCR band intensity results showing differential expression in two OsGLK genes.

However, *K37* is reported earlier to be up-regulated in roots of LR 26 under 15-days low P condition (Tyagi *et al.*, 2017). Root transcriptome data also reported the differential expression of three other *GLK* family members namely *K8*, *K10* and *K19* (Table 4.11).

Table 4.11 List of *GLK* genes identified from root transcriptome dataset and semi quantitative PCR showing differential expression under low P conditions

OsGLK members identified from root transcriptome data (Tyagi <i>et al.</i> , 2017)			
Transcript_ID	Gene name	Genotypes	Regulation
LOC_Os02g07770	<i>K8</i>	LR 23	Down
LOC_Os02g22020	<i>K10</i>	Both LR 23 & LR 26	Up
LOC_Os04g47890	<i>K19</i>	LR 26	Up
LOC_Os08g33050	<i>K37</i>	LR 26	UP
OsGLK member identified from semi quantitative PCR			
LOC_Os03g20900	<i>K14</i>	LR 23	Up
LOC_Os08g33050	<i>K37</i>	LR 26	Down

4.6 Quantitative/real time PCR

A total of 13 *GLK* genes including the differentially expressing genes under low P conditions identified from root transcriptome data and semi quantitative PCR, plus the members of *GLK* carrying multiple copies of P1BS cis-acting elements (Table 4.10) were subjected to real time PCR.

Ten genes are found to be differentially expressing under low P conditions. Gene *K4* is up-regulated in susceptible genotype (BAM 350), *K41* is down-regulated in BAM 350, *K8* is up-regulated both in tolerant genotype (LR 26) and susceptible genotype (BAM 811), *K7* is down-regulated in LR 26, *K19* is up-regulated in LR 23 and BAM 350, *K30* is down-regulated in tolerant genotype (LR 26) and up-regulated in susceptible genotype (BAM 350), *K15* is down-regulated in LR 23, *K14* is up-regulated in LR 23 and BAM 350 but down-regulated in BAM 811, *K37* is up-regulated in LR 23 and BAM 350 and *K10* is up-regulated in both the tolerant genotypes (LR 23, LR 26) and down-regulated in both the susceptible genotypes (BAM 350 and BAM 811) (Fig. 4.8 and Fig. 4.9). RT primer belonging to gene *K3* did not work.

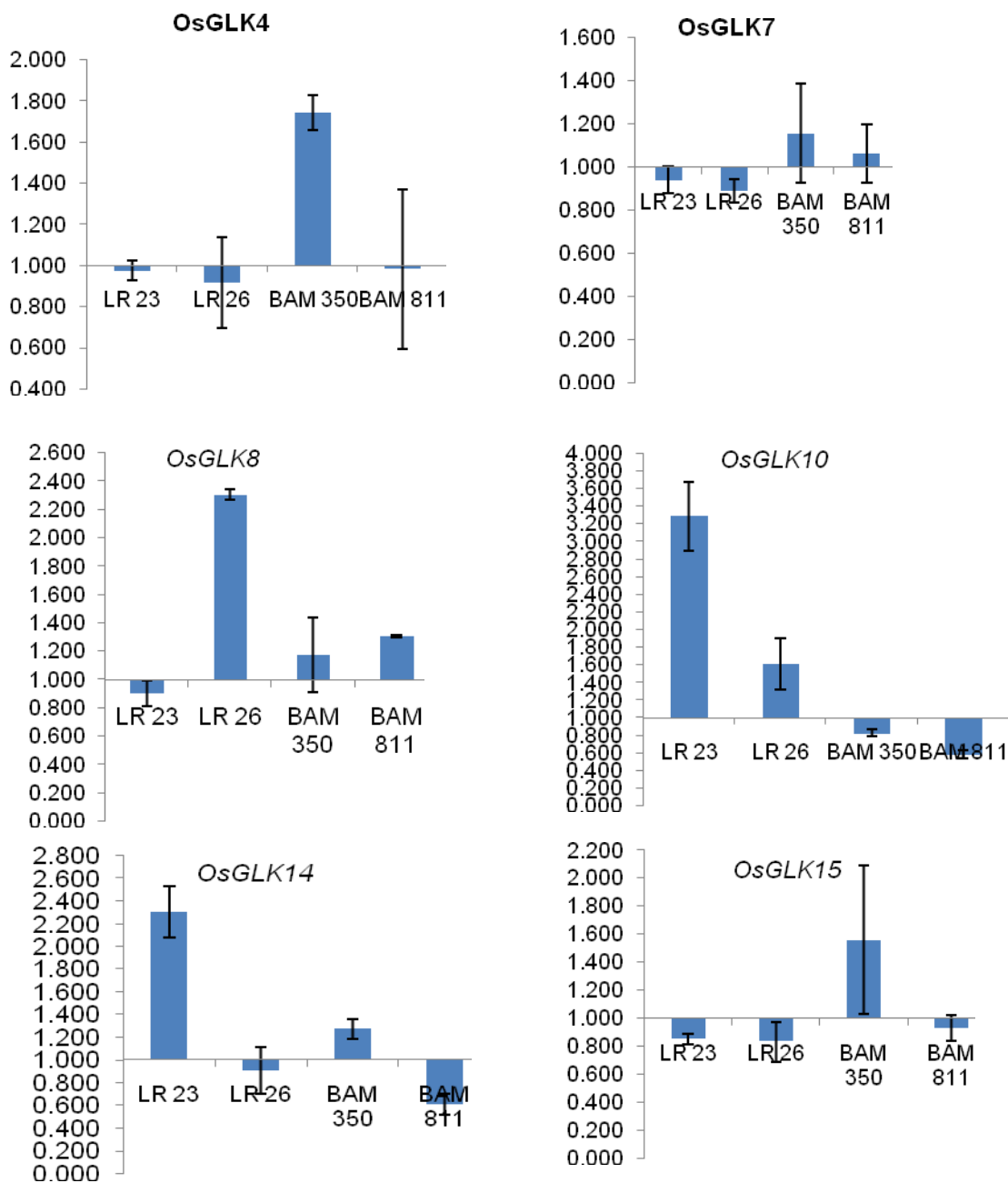


Fig. 4.8 Graphical representation of six *OsGLK* gene family members showing differential expression under P deficiency condition in tolerant (LR 23 and LR 26) and susceptible (BAM 350 and BAM 811) rice genotypes (Y axis represents log₂ fold expression level). The name of the gene is indicated at the top. The datum is average of two technical replicates.

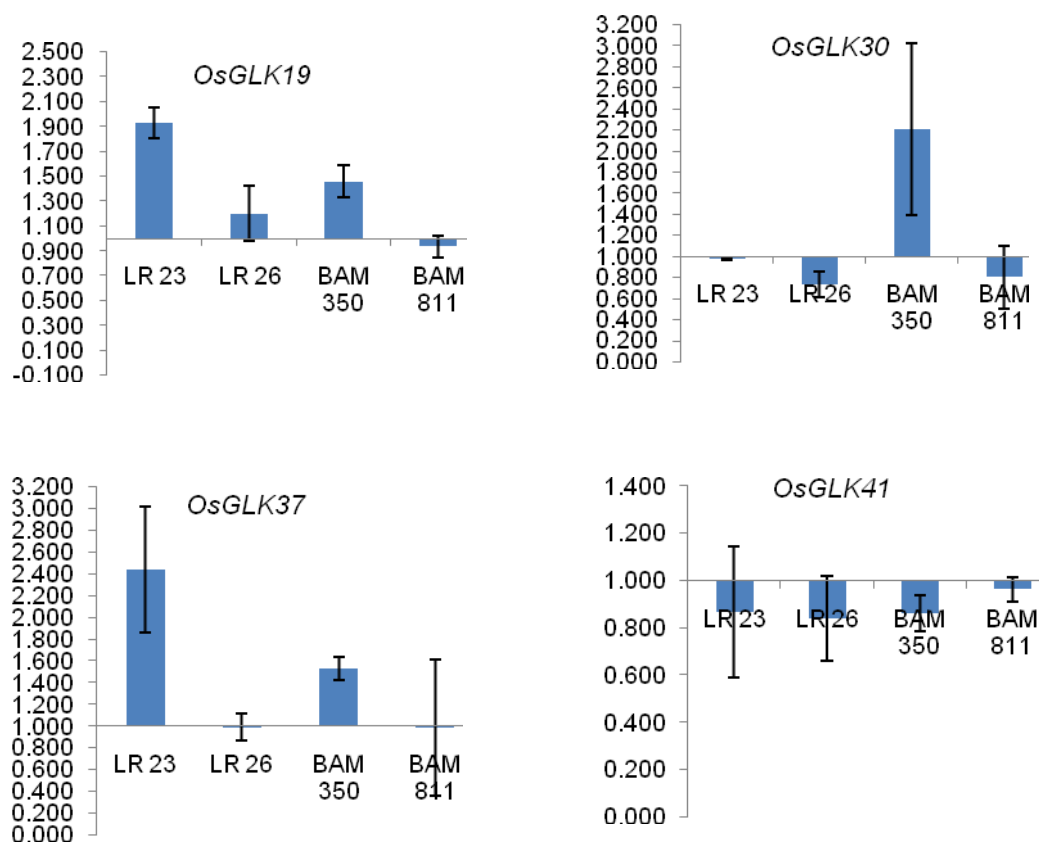


Fig. 4.9 Graphical representation of four *OsGLK* gene family members showing differential expression under P deficiency condition in tolerant (LR 23 and LR 26) and susceptible (BAM 350 and BAM 811) rice genotypes (Y axis represents log₂ fold expression level). The name of the gene is indicated at the top. The datum is average of two technical replicates.

4.7 Field evaluation of advanced breeding lines under lowland acidic soil conditions

RILs generated by crossing LR 23 and LR 26 showed wide range of phenotypic variation (Fig. 4.10 and 4.11).

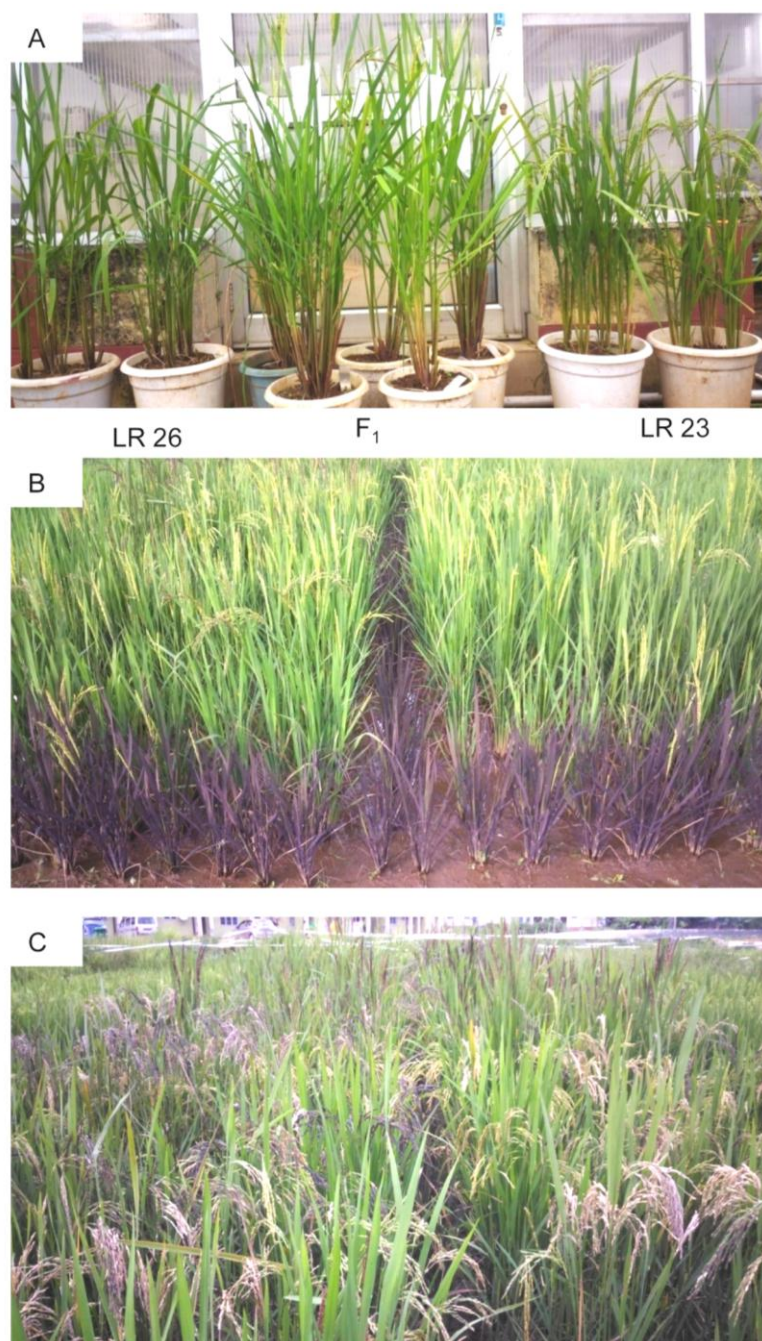


Fig. 4.10 Phenotype of plants a) two parental lines and F₁ b) RILs at flowering time c) RILs at maturity



Fig. 4.11 A representative photograph depicting phenotypic variation in RILs derived from LR 23 and LR 26

Data collected from five plants of each line were subjected to statistical analysis and the results are as follows.

4.7.1 Correlation and frequency distribution analysis of the traits for yield

Data on five plants/line for three subsequent years (2015, 2016 and 2017) were taken from population grown under lowland acidic field and normalized with help of software ABDC-R. Correlation between the traits was calculated from the normalized field data of each year to identify the major traits that were directly related with yield of the plants (Table 4.12, 4.13 and 4.14). Correlation studies on data collected in the year 2015 from F_4 population showed significant correlation between the traits like PH with PL (0.632), TN60 with FG (0.6) and FG with F % (0.56) (Table 4.12).

Similarly, correlation studies on data collected in the year 2016 from F_5 population showed significant correlation between traits like TN 30 with TN 60 (0.62), PN (0.514) and TN 60 with PN (0.9) and PDW (0.656) (Table 4.13). Trait PDW was significantly correlated with PN (0.71) and FG (0.559) and FG with %F (0.664).

Correlation studies on data collected in the year 2016 (Table 4.14) from F_6 population showed significant correlation between traits like TN 30 with TN 60 (0.779), PN (0.578), FG (0.517), GYPP (0.53), PDW at harvest (0.6) and PUE at harvest (0.564). TN 60 with PN (0.809), FG (0.692), GYPP (0.697), PDW at harvest (0.73) and PUE at harvest (0.663). Trait PN was correlated with FG (0.757), GYPP

(0.737), PDW at harvest (0.799) and PUE at harvest (0.732). Trait GYPP was correlated with PDW at harvest (0.857) and PUE at harvest (0.755). PDW at 60 DAT was correlated with PUE at 60 DAT (0.922). PDW at harvest was significantly correlated with PUE at harvest (0.891).

Table 4.12 Correlation between six yield related traits as observed in 2015 lowland field for F₄ plants.

Traits	TN 60	PL(cm)	PDW harvest (g)	FG	F%	100 SW (g)
PH (cm)	0.457	0.623*	0.252	0.4	0.04	-0.002
TN	1	0.409	0.385	0.6*	0.07	0.045
PL (cm)		1	0.229	0.4	0.08	0.127
PDW (g)			1	0.3	-0.05	0.015
FG				1	0.56*	-0.023
F %					1	0.07

where PH=plant height; TN60= tiller number at 60 days; PL= Panicle length; FG= filled grains per plant; F %= spikelet fertility %; 100 SW= 100 seed weight. Significant values are indicated by ‘*’.

Table 4.13 Correlation between nine yield related traits as observed in 2016 lowland field for F₅ plants.

Traits	TN 60	50 %DTF	PN	PH (cm)	PL (cm)	PDW (g)	FG	F %	100 SW
TN 30	0.62*	-0.182	0.514*	0.148	-0.01	0.385	0.136	-0.062	0.005
TN 60	1	-0.038	0.9*	0.094	0.025	0.656*	0.328	-0.065	0.016
50% DTF		1	0.07	0.334	0.13	0.363	0.052	-0.144	-0.102
PN			1	0.165	0.08	0.71*	0.413	-0.024	0.048
PH (cm)				1	0.368	0.466	0.144	-0.109	-0.115
PL (cm)					1	0.288	0.231	0.071	0.106
PDW (g)						1	0.559*	0.085	0.106
FG							1	0.664*	0.233
F %								1	0.366

Note: TN30 = tiller number at 30 days after transplanting; TN60 = tiller number at 60 days after transplanting; 50 % DTF = days to 50 % flowering; PN = panicle number per plant; PL= panicle length; PH = plant height; PDW =plant dry weight, F % = spikelet fertility %; FG = number of filled grains per plant; 100 SW = 100 seed weight. Significant values are indicated by ‘*’.

Table 4.14 Correlation between fifteen yield related traits as observed in 2017 lowland field for F₆ plants.

TRAITS	TN 60	50% DTF	PH	PL	LA cm ²	PN	FG	100 SW	F %	GYPP	P content	PDW (Harvest)	PUE at harvest	PDW 60 DAT	PUE at 60
TN30	0.779*	-0.057	0.248	0.171	-0.091	0.578*	0.517*	-0.032	0.185	0.53*	0.02	0.6*	0.564*	0.318	0.296
TN60	1	0.184	0.22	0.059	-0.015	0.809*	0.692*	0.09	0.26	0.697*	0.036	0.73*	0.663*	0.309	0.27
50%DTF		1	0.361	0.352	0.055	0.321	0.271	-0.009	0.051	0.229	0.136	0.356	0.245	0.102	0.029
PH (cm)			1	0.352	0.057	0.269	0.292	-0.065	0.079	0.274	0.037	0.486	0.424	0.287	0.269
PL (cm)				1	-0.098	0.413	0.385	0.249	0.054	0.375	-0.063	0.465	0.425	0.225	0.213
LA cm ²					1	-0.047	0.034	-0.304	0.122	0.053	-0.068	0.001	0.012	-0.021	-0.005
PN						1	0.757*	0.205	0.262	0.737*	0.012	0.799*	0.732*	0.243	0.194
FG							1	0.054	0.59*	0.962*	0.035	0.878*	0.771*	0.323	0.268
100 SW								1	-0.061	0.062	-0.138	0.04	0.079	-0.144	-0.111
F %									1	0.602	-0.036	0.341	0.334	0.136	0.138
GYPP										1	0.02	0.857*	0.755*	0.332	0.281
P content											1	0.063	-0.321	0.105	-0.215
PDW harvest												1	0.891*	0.403	0.336
PUE harvest													1	0.306	0.386
PDW 60														1	0.922*

Note: TN30 =tiller number at 30 days; TN60 = tiller number at 60; 50%DTF = days to 50 % flowering; PN =panicle number; PL=panicle length; PH =plant height; PDW =plant dry weight, F % = spikelet fertility %; FG = number of filled grains per plant; 100 SW = 100 seed weight; GYPP = grain yield per plant; PUE = phosphorus use efficiency; LA = leaf area. Significant values are indicated by ‘*’.

Number of traits for which data were collected in three years increased every year, because the traits were added to identify significantly correlated traits in non-segregating generations. Through correlation analysis of three years field data, traits like TN30, TN60, FG, F %, PN and GYPP were found to be significantly correlated with each other and were associated with better yield under low P acidic soil conditions.

On the basis of 5 traits as mentioned in materials and method, 40 good performing lines and 40 bad performing lines were identified (Table 4.15) for selective genotyping.

Table 4.15 List of good and bad performing lines identified for selective genotyping

Good performing lines				Bad performing lines			
Sl. No.	Genotypes	Sl. No.	Genotypes	Sl. No.	Genotypes	Sl. No.	Genotypes
1	230(3)-1	21	222(3)-5	41	161(3)-1	61	19(4)-1
2	154(2)-1	22	33(1)-1	42	87(3)-4	62	50(4)-1
3	198(2)-1	23	188(4)-5	43	120(4)-2	63	117(5)-1
4	195(5)-3	24	102(4)-4	44	29(1)-3	64	41(5)-5
5	132(2)-1	25	17(3)-4	45	60(3)-4	65	224(1)-3
6	61(1)-2	26	11(4)-1	46	90(1)-2	66	180(2)-1
7	18(2)-1	27	34(3)-4	47	62(4)-4	67	171(2)-1
8	80(1)-1	28	152(5)-1	48	156(4)-2	68	42(3)-2
9	95(4)-1	29	122(5)-5	49	37(4)-5	69	196(5)-1
10	90(5)-5	30	215(2)-4	50	49(1)-3	70	2(4)-3
11	122(5)-1	31	229(4)-3	51	51(4)-3	71	165(2)-4
12	73(4)-1	32	172(3)-3	52	23(3)-3	72	93(1)-2
13	8(4)-4	33	59(4)-4	53	48(3)-2	73	124(1)-1
14	153(4)-5	34	22(2)-4	54	168(4)-3	74	123(1)-2
15	206(5)-5	35	163(1)-5	55	55(2)-3	75	221(1)-5
16	220(2)-4	36	114(2)-4	56	7(3)-1	76	78(1)-1
17	181(4)-2	37	81(4)-3	57	235(1)-2	77	170(1)-5
18	201(2)-2	38	193(1)-1	58	205(4)-2	78	133(1)-1
19	83(4)-2	39	34(3)-1	59	36(4)-3	79	192(4)-4
20	87(4)-2	40	65(3)-5	60	196(4)-1	80	46(3)-2

Frequency distribution of six traits (TN30, TN60, PN, F%, FG and 100 SW) based on which 40 good and 40 bad performing lines selected are given in Fig. 4.12.

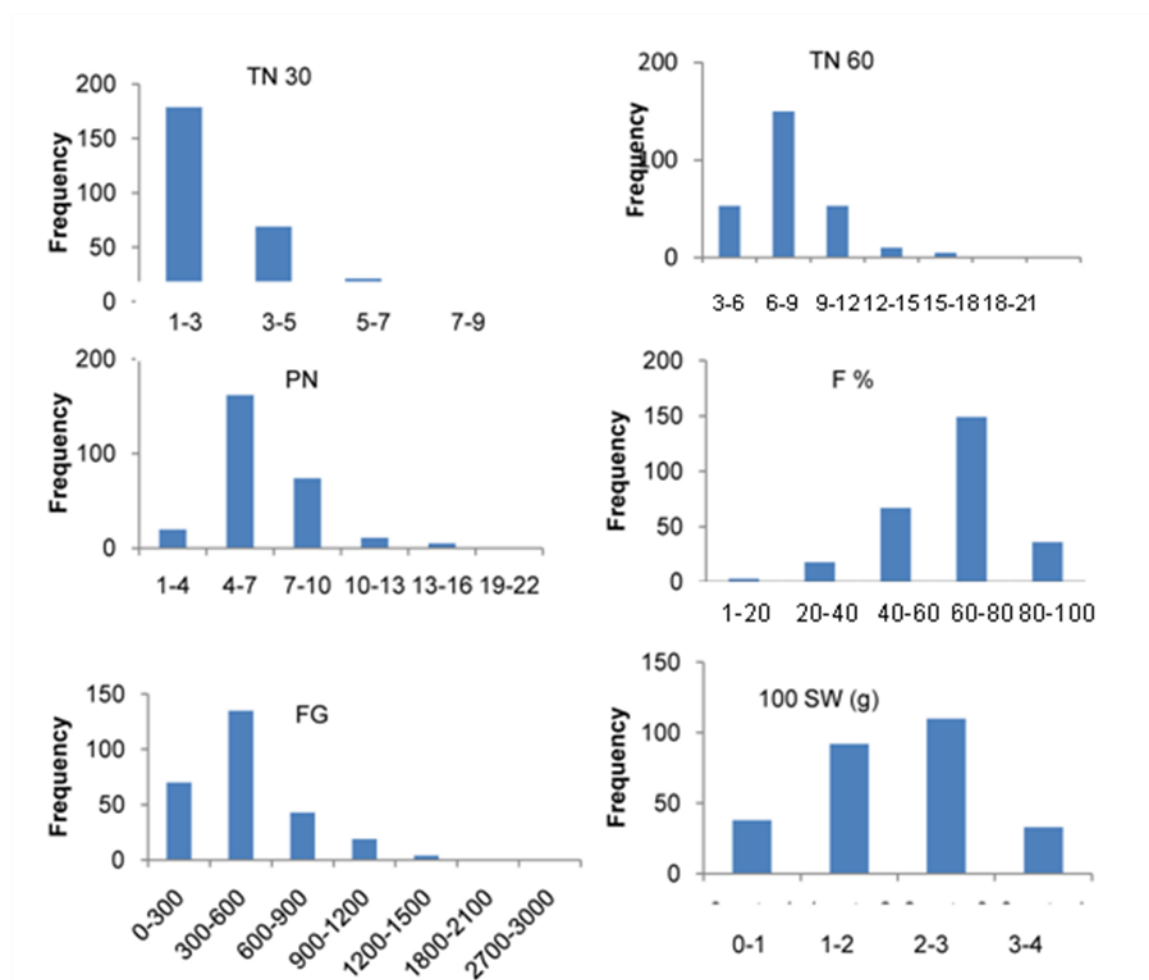


Fig. 4.12 Frequency distribution of six traits (2017) in the selected 80 lines identified from a total of 357 F_6 lines

After normalizing data of 5 plants from each line, an average value for each line was calculated and used for plotting frequency distribution. The distribution of TN 30 ranged from 1-5 tillers with 1-3 tillers having maximum frequency (#180) (Fig. 4.12). Minimum value was obtained for >7 tillers. Frequency of TN 60 ranged from 4-11 tillers. Number of filled grains ranged from 99-1100 per plant. The fertility % ranged from 31-98% with maximum frequency found for 300-600 range and minimum for >1800 grains. The distribution of 100 SW ranged from 0.13-3.52 g.

4.7.2 Phenotypic distribution of yield attributing traits in selected good and bad performing lines

The phenotypic data of 40 good performing lines and 40 bad performing lines showed differences in their mean value (Fig. 4.13 and Fig. 4.14) especially for the traits like TN 30, TN 60, PN, F %, GYPP, BY/PDW at harvest, PUE harvest and PUE at 60 DAT.

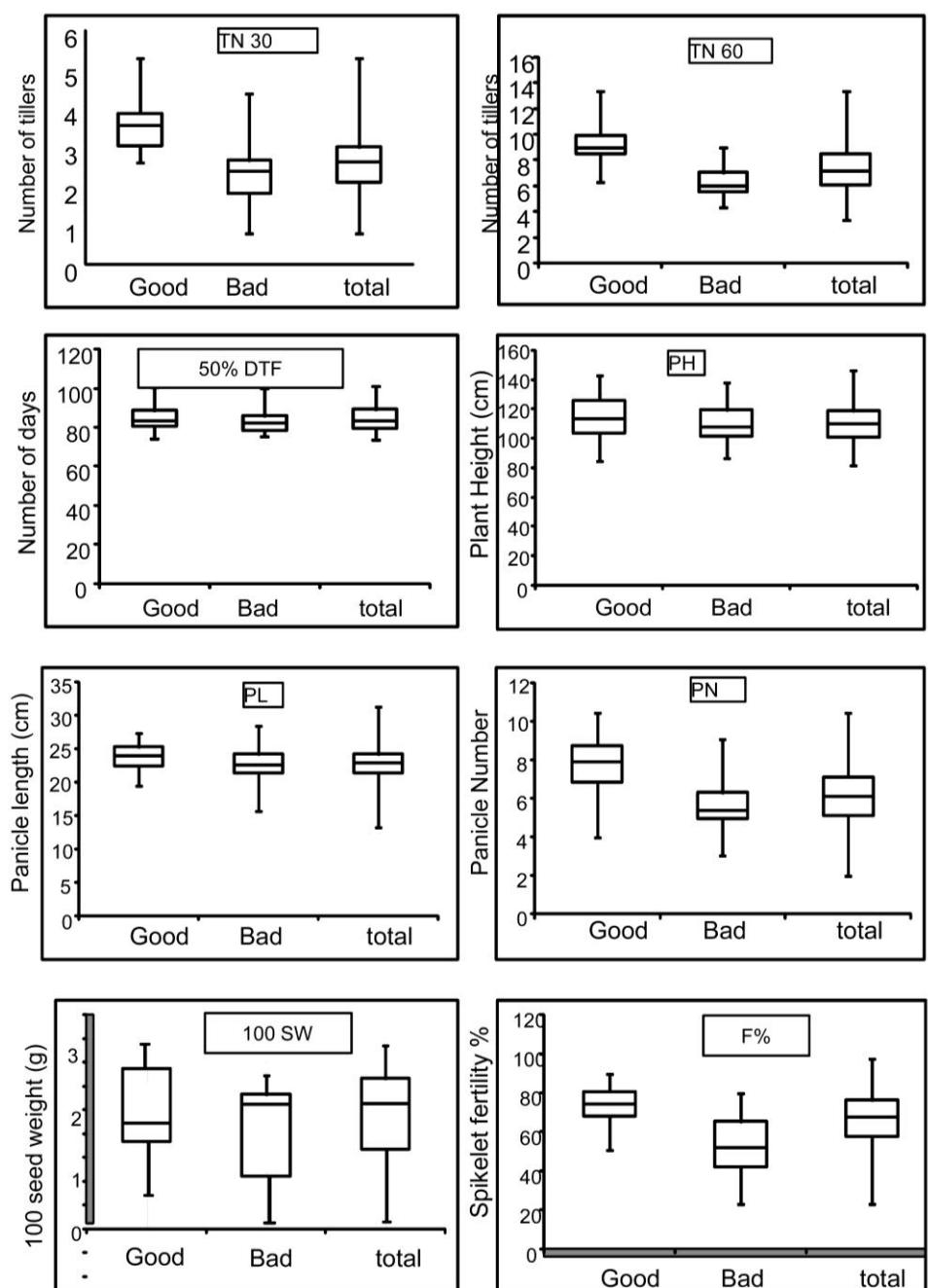


Fig. 4.13 Box-plot distribution for eight traits for 80 good and bad performing lines

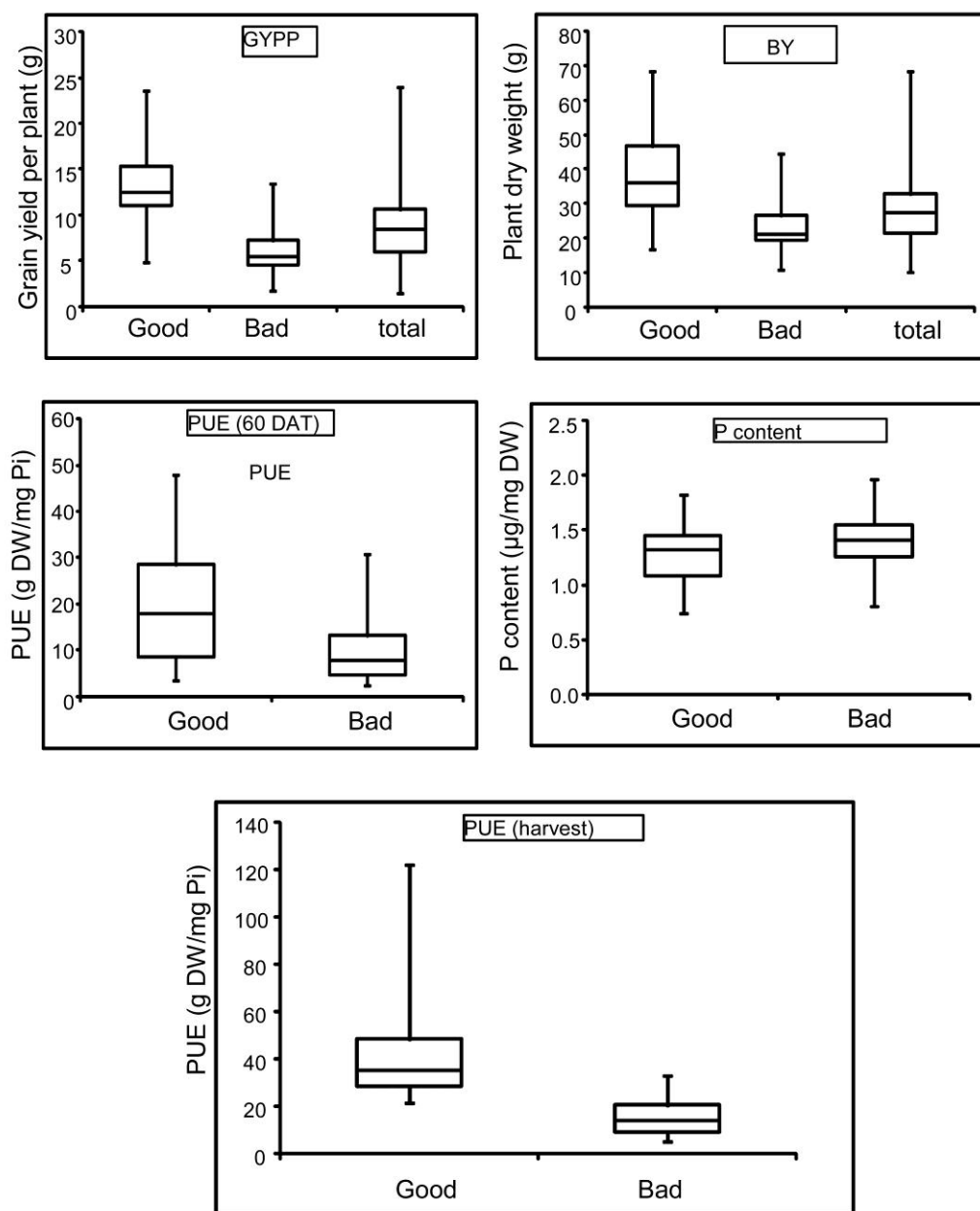


Fig. 4.14 Box-plot distribution for five traits for 80 good and bad performing lines

4.8 Parental polymorphism survey with molecular markers

From a total of 784 markers used for polymorphism survey thirty one candidate gene based markers belonging to fourteen genes including *GLK* and *PSTOL1* gene (Fig. 4.16), twenty five SSR marker including four genic SSR markers (HvSSR02-10, 04-49, 10-30 and 11-13) (Fig. 4.15) and eighteen SNPs were identified as polymorphic .

The detail list of markers is given in annexure 2. A total of 31 polymorphic candidate gene based markers, 14 markers (one from each gene) and all the polymorphic SSR and SNPs were selected for genotyping the panel of 80 genotypes. Polymorphic markers were found to be distributed on all the 12 chromosomes of rice with maximum of 12 markers on chromosome 2, seven on chromosome 6, six markers on chr #12, five markers each on chr # 3 and chr #11, four markers each on chr #4, chr #5 and chr #10, three markers each on chr #1 and chr #8 and one marker on chr #9 (Fig. 4.17).

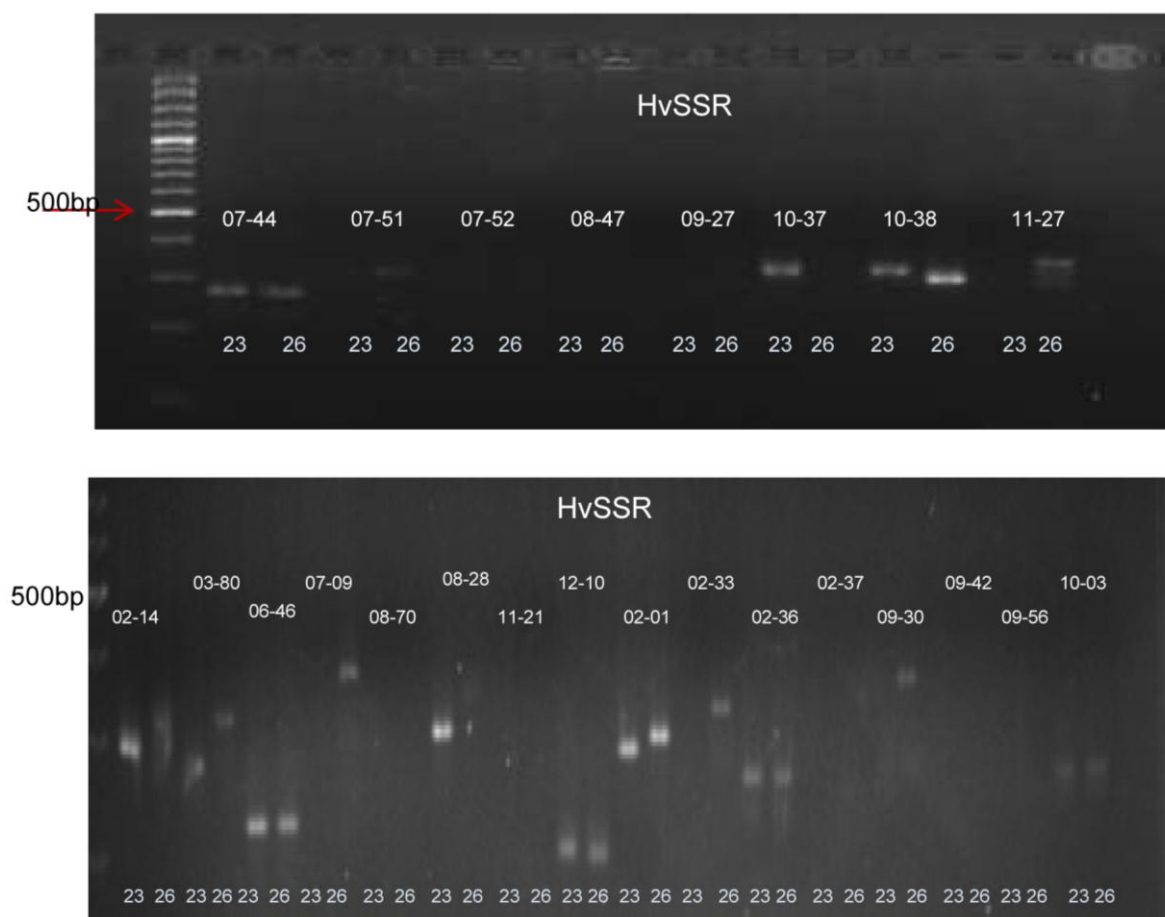


Fig. 4.15 Parental polymorphism survey using hypervariable SSR (HvSSR) markers. The name of the markers is indicated at the top of the gel. Numbers 23 and 26 indicate LR 23 and LR 26, respectively.

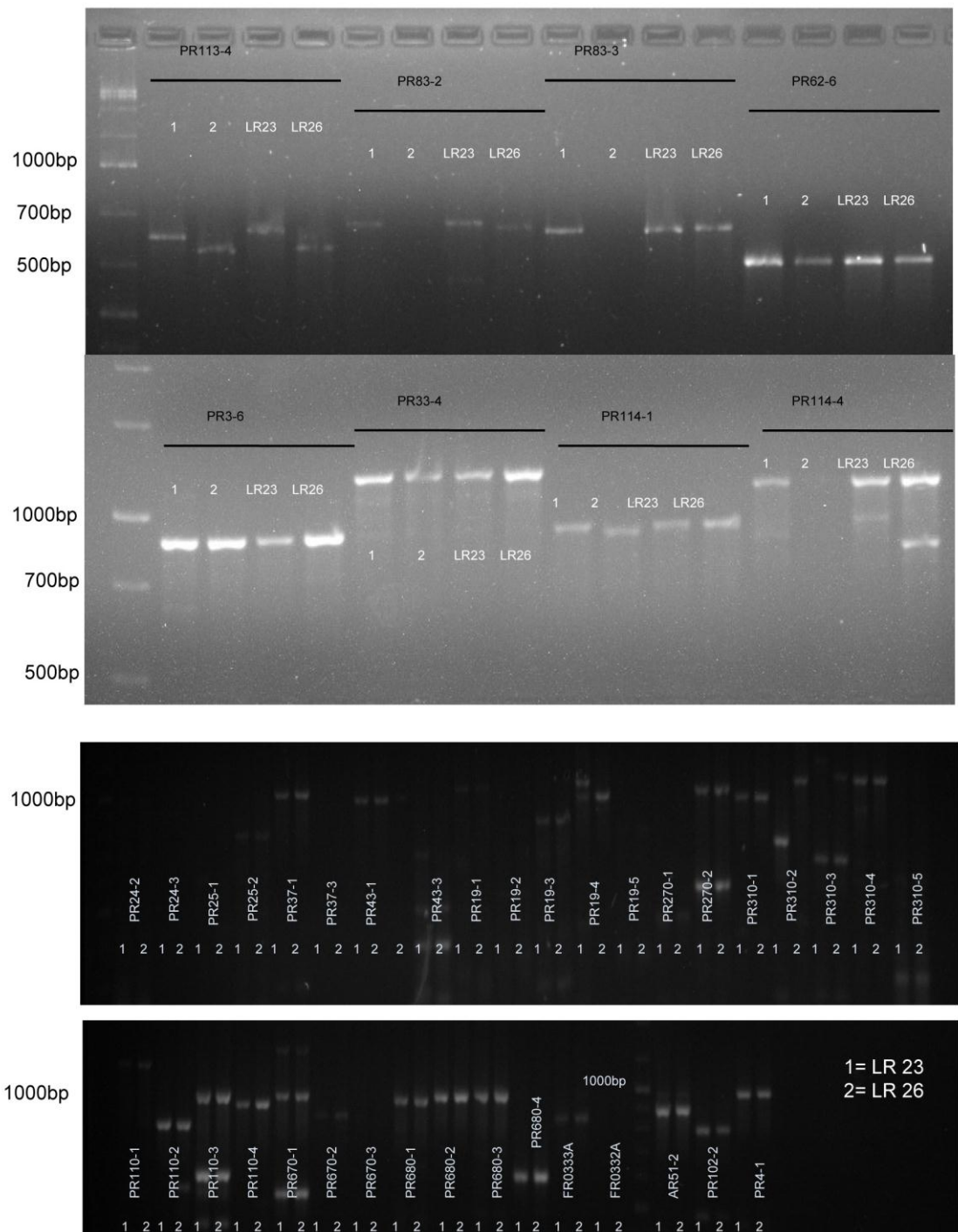


Fig. 4.16 Parental polymorphism survey using candidate gene based markers. The name of the markers is indicated at the top/bottom, and 1 and 2 indicate LR 23 and LR 26, respectively in the gels in lower panel.

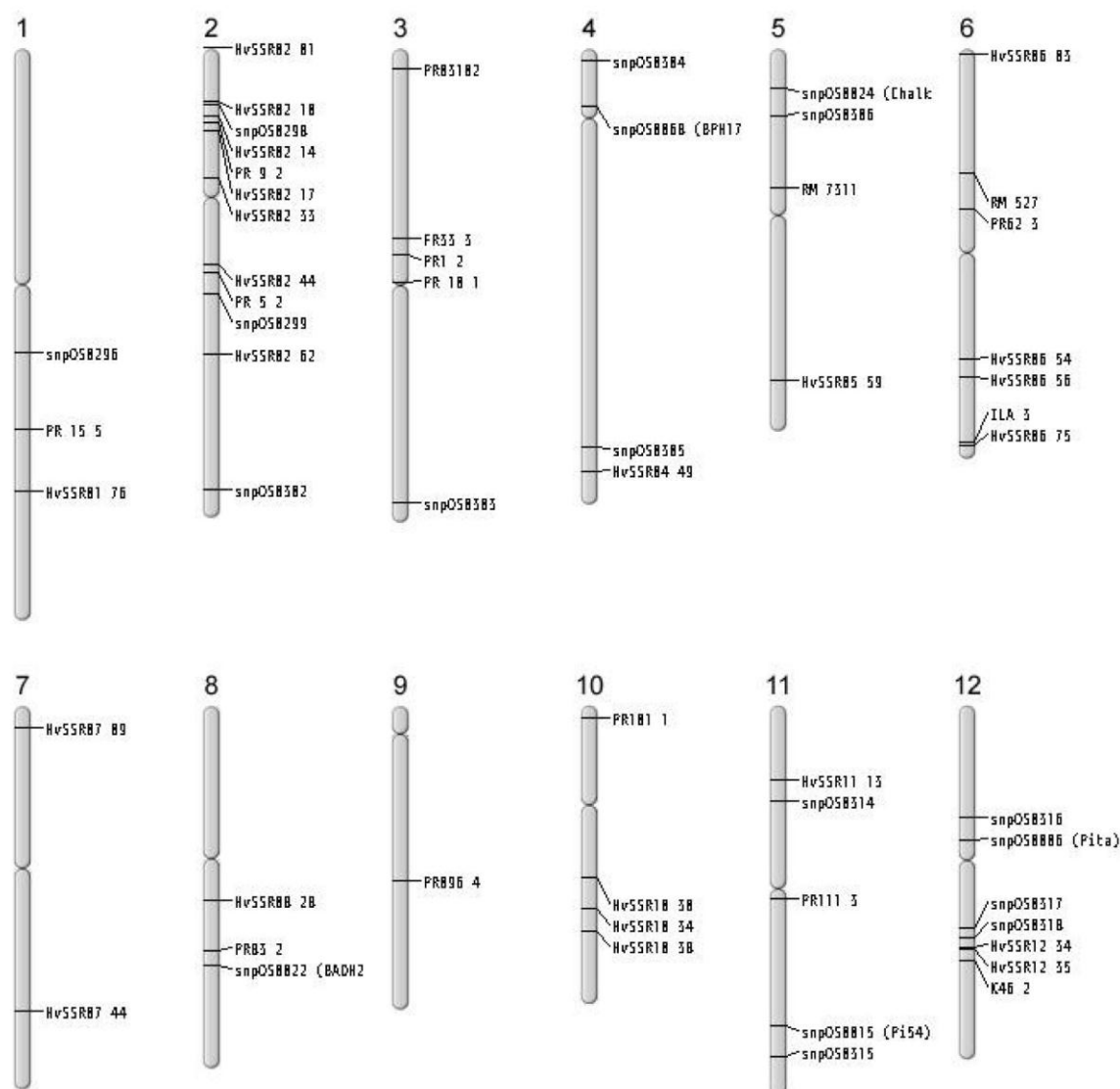


Fig. 4.17 Chromosomal map of the polymorphic markers between LR 23 and LR 26 shown on the 12 rice chromosomes.

4.9 Selected genotyping using polymorphic markers

A total of 58 polymorphic markers are used for genotyping the selected extreme lines. Gel based polymorphism for candidate gene based markers and SSR markers is done by using 1.5 and 2.5 % agarose gel, respectively (Fig 4.18, 4.19, 4.20).

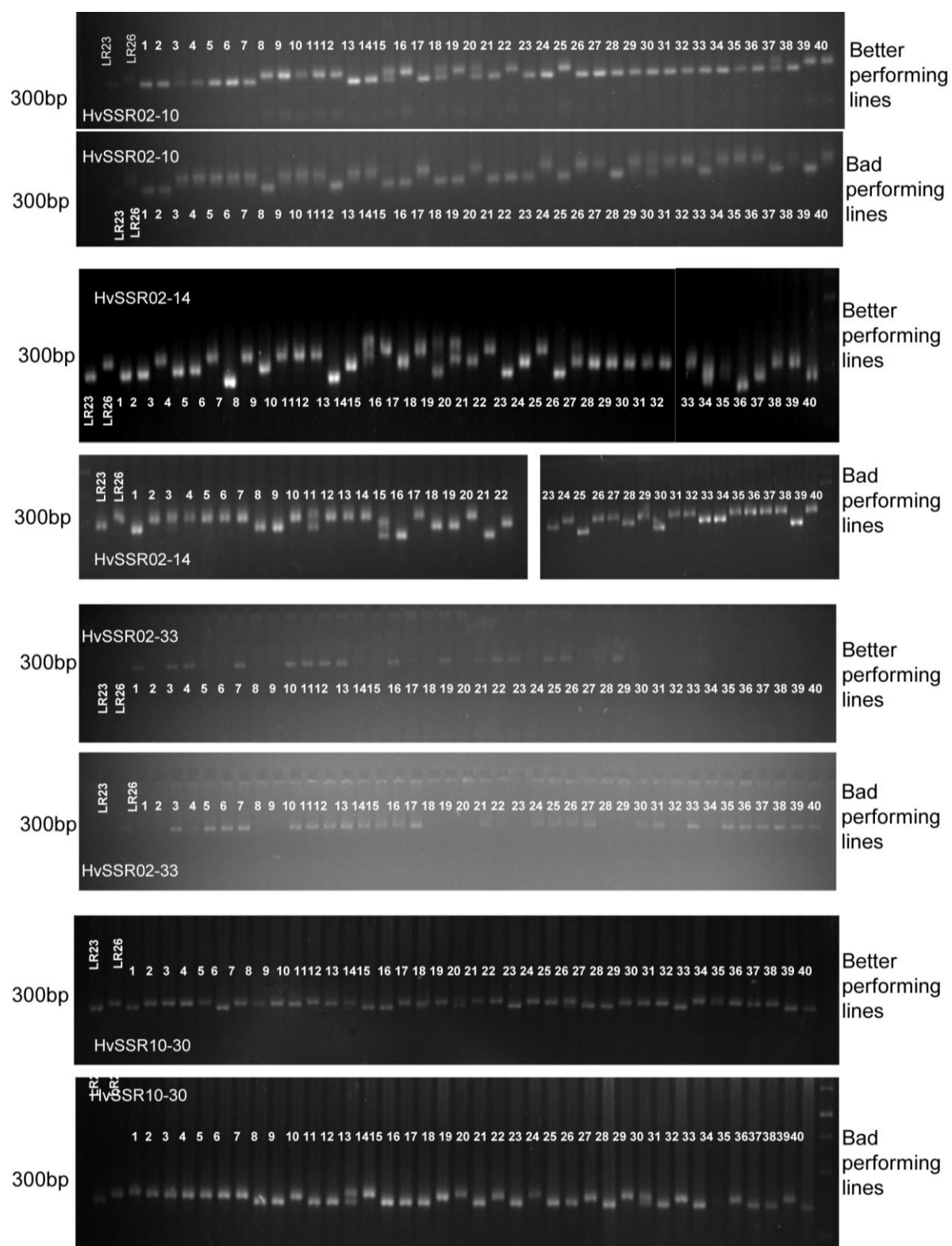


Fig. 4.18 Amplification profile of SSR markers HvSSR02-10, HvSSR02-14, HvSSR02-33 and HvSSR10-30 on a panel of 80 rice genotypes selected from 357 F₆ RIL for contrasting response in terms of yield parameters under acidic soils. The name of the markers is indicated at the top/bottom of the gel. Numbers 1-40 for the better and bad performing lines represent the selected line as mentioned in table 4.15.

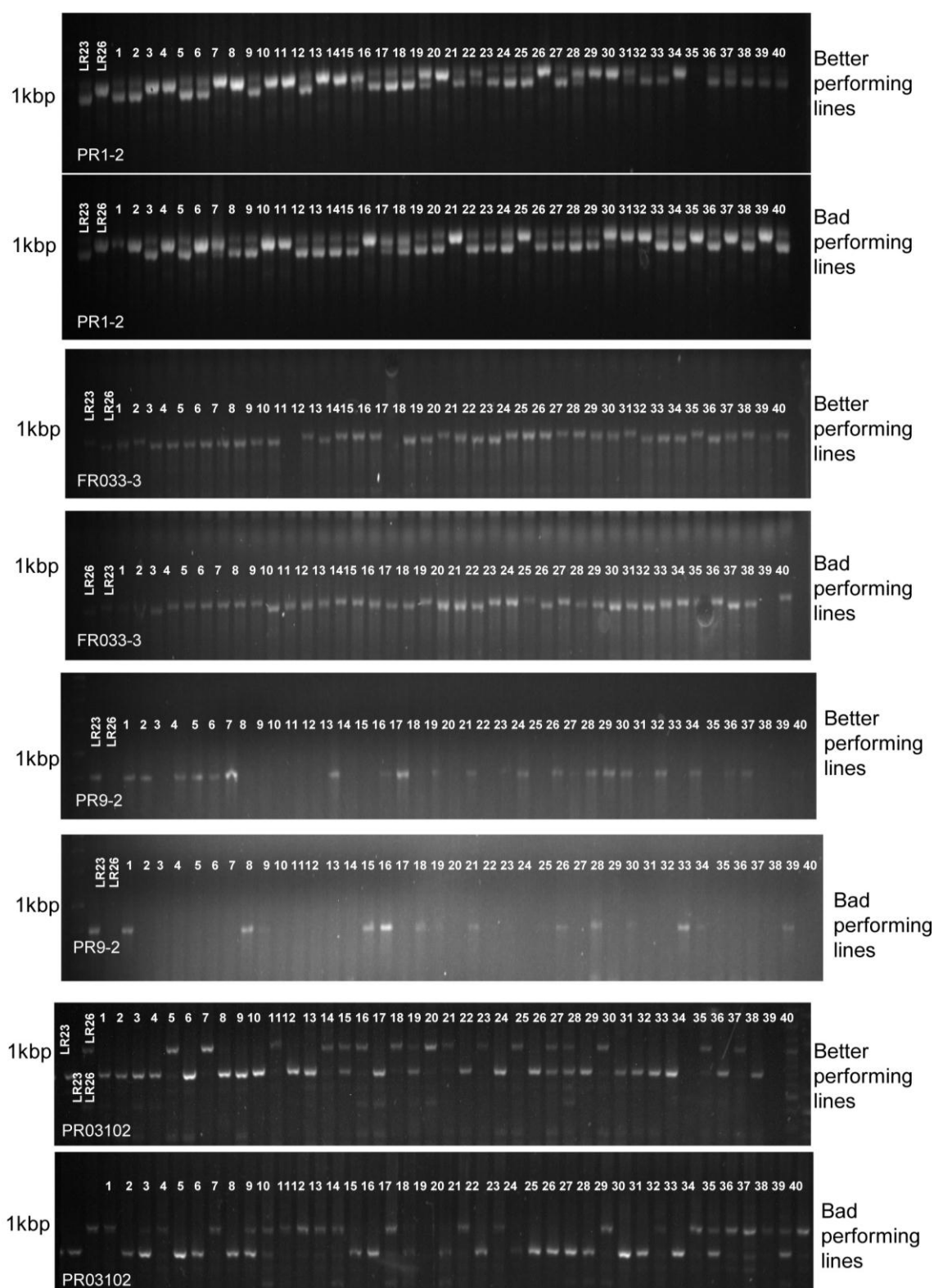


Fig. 4.19 Amplification profile of candidate markers PR1-2, FR033-3, PR9-2 and PR03102 on a panel of 80 rice genotypes selected from 357 F_6 RIL for contrasting response in terms of yield parameters under acidic soils. The name of the markers is indicated at the top/bottom of the gel. Numbers 1-40 for the better and bad performing lines represent the selected line as mentioned in table 4.15.

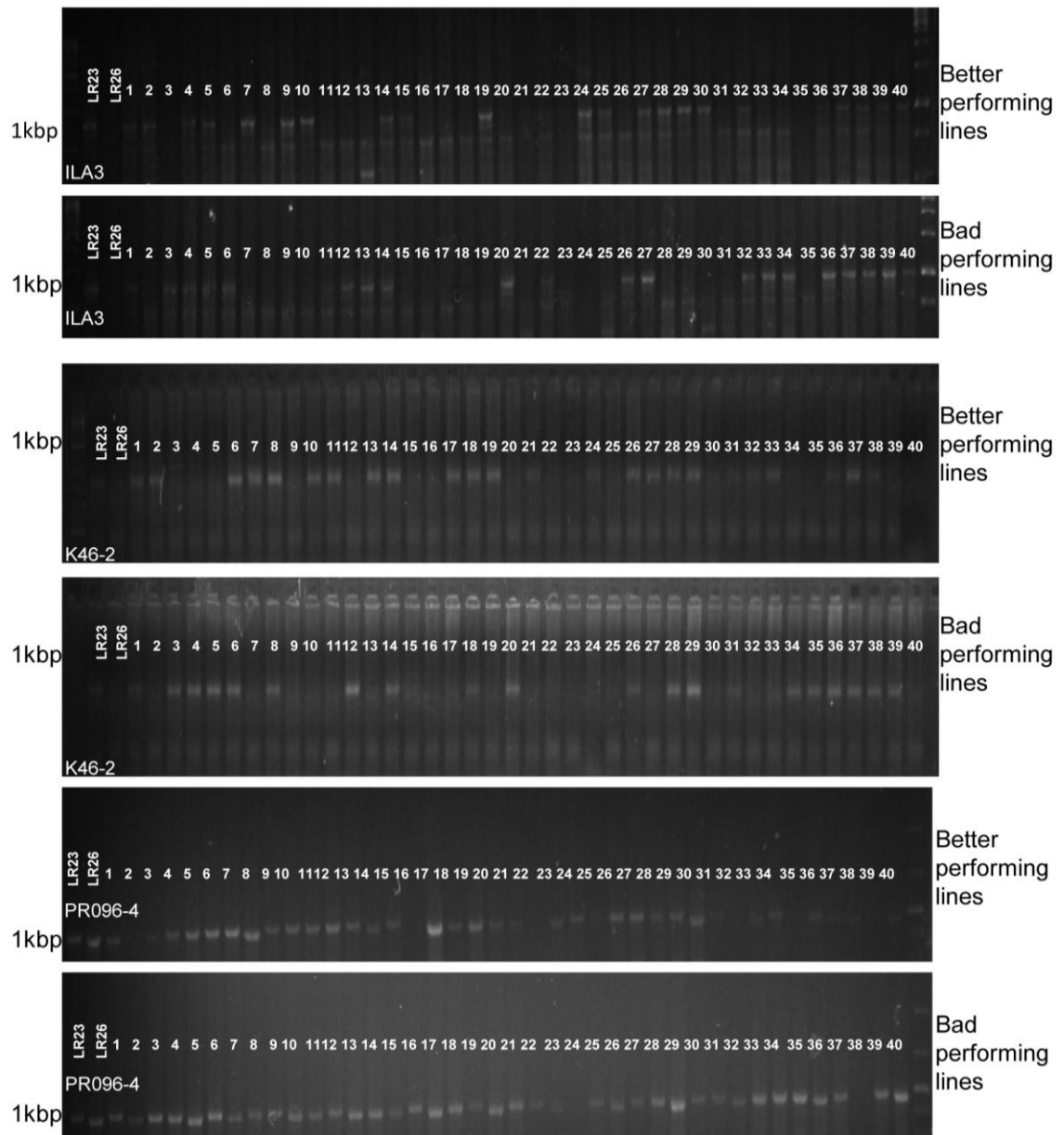


Fig. 4.20 Amplification profile of candidate markers ILA3, K46-2 and PR096-4 on a panel of 80 rice genotypes selected from 357 F₆ RIL for contrasting response in terms of yield parameters under acidic soils. The name of the markers is indicated at the top/bottom of the gel. Numbers 1-40 for the better and bad performing lines represent the selected line as mentioned in Table 4.15.

4.10 Marker trait association studies

4.10.1 Chi square test results

After scoring genotypic data for each polymorphic marker on selected good and bad lines, chi square analysis was done (Table 4.16). In chi square analysis, out of 58 polymorphic markers used, 14 markers showed significant association at $P=0.05$ level. PR9-2 is associated with FG ($\chi^2=6.667$), F % ($\chi^2=5.013$), GYPP ($\chi^2=6.667$), PUE at 60 DAT ($\chi^2=8.12$). SSR marker HvSSR02-10 associated with FG ($\chi^2=4.428$), F % ($\chi^2=10$), GYPP ($\chi^2=9.33$), PUE at harvest ($\chi^2=4.9$) and PUE at 60 DAT ($\chi^2=5.803$). Marker HvSSR02-14 associated with FG ($\chi^2=9.608$), F % ($\chi^2=10.68$), GYPP ($\chi^2=8.67$), PUE at harvest ($\chi^2=6.951$) and PUE at 60 DAT ($\chi^2=7.725$). HvSSR02-33 associated with FG ($\chi^2=3.95$), GYPP ($\chi^2=5.227$) and PUE at harvest ($\chi^2=4.285$). Markers HvSSR10-30 and 34 associated with PN ($\chi^2=5.95$ and 5.657), respectively. HvSSR07-44 associated with F % ($\chi^2=3.956$), FR033-3 associated with FG ($\chi^2=4.286$), PR1-2 associated with TN 60 ($\chi^2=3.956$), PR096-4 associated with PUE at 60 DAT ($\chi^2=5.253$), ILA3 associated TN 60 ($\chi^2=4.912$), K46-2 associated with PUE at harvest ($\chi^2=4.912$), snpOS305 associated with F % ($\chi^2=6.474$) and GYPP ($\chi^2=9.218$), snpOS316 associated with TN 30 ($\chi^2=4.244$), TN 60 ($\chi^2=4.343$) and GYPP ($\chi^2=4.161$). For almost all the markers, LR 23 type allele is associated with better performance and LR 26 type allele with poor performance, except for markers FR033-3 and PR1-2 and HvSSR07-44, where LR 23 type allele is associated with inferior performance. In case of HvSSR07-44, LR 26 type allele is associated with superior performance (Table 4.16).

Table 4.16 Chi square value (significant values are marked red)

Markers	Ch. no.	TN 30	TN 60	PN	FG	100 SW	F %	GYPP	PUE (g DW/mg Pi)	60 days PUE (g DW/mg Pi)
HvSSR01-76	1	3.6	0.4	0	0.4	2.506	3.75	0.404	3.636	0.9
PR 15-5	1	0.902	0.1	0.1	0.1	0.106	0.404	0.1	1.6	0.902
snpOS0296	1	0.81	0.805	0.148	1.138	2.867	2.575	2.251	0.148	0.306
PR 9-2	2	0.902	0.102	0.404	6.667 **	2.666	5.013 **	6.667 **	2.666	8.12 **
HvSSR02-01	2	0.92	0.104	0.025	0	0.228	0.025	0.416	0.102	0
HvSSR02-10	2	2.667	1.637	2.525	4.428 **	0.243	10 **	9.33 **	4.9 **	5.803 **
HvSSR02-14	2	3.113	0.701	2.622	9.603 **	3.459	10.68 **	8.67 ^-	6.951 **	7.725 **
HvSSR02-17	2	1.264	0.243	0.028	2.506	3.6	3.75	3.027	0.404	0.625
HvSSR02-33	2	0.102	0.102	2.557	3.95 ^-	0.113	2.666	5.227 ^-	4.285 ^-	0.404
HvSSR02-44	2	0.628	0.634	0.025	0.676	1.231	0.658	1.29	1.325	1.666
HvSSR02-62	2	0.274	2.264	1.758	0.416	0.251	2.19	0.902	0.439	0.426
snpOS0298	2	0	0.003	0.098	0.006	0	0	0	0.011	0.002
snpOS0299	2	0.064	0.114	0.082	0.009	0.023	0.079	0.004	0.063	0.141
snpOS0302	2	0.182	0.251	0.944	1.111	1.078	0.891	1.11	0.019	0.977
PR 5-2	2	0	0	0.1	2.666	0.1	1.666	2.666	0	0.113
PR10-1	3	0.135	1.944	0.008	0.068	0.468	0.211	0.003	1.084	0.244
PR03102	3	1.049	0.671	0.496	0.528	0.912	3.229	0.791	0.342	1.226
PR03102	3	1.049	0.671	0.496	0.528	0.912	3.229	0.791	0.342	1.226
FR 033-3	3	1.904	1.128	0.96	4.286 *-	0.113	1.129	1.128	0.106	0.902
PR1-2	3	0.229	3.956 *-	0	0.902	0.629	0.025	0.1	2.036	0.629
snpOS0303	3	0.043	0.109	0.121	0.118	2.941	0.772	0.791	0.058	0.106
HvSSR04-49	4	0.226	0.644	0.109	0.698	1.263	1.6	0.439	2.19	2.036
snpOS0068	4	0.625	1.023	1.198	0.4	0.446	0.029	0.079	0.171	0.335
snpOS0304	4	0.245	0.459	2.224	3.339	0.104	0.117	1.517	0.267	1.481
snpOS0305	4	1.47	2.098	0.121	0.164	0.718	6.474 **	9.281 **	1.557	0.723
HvSSR05-59	5	1.616	0.628	0.1	0.1	2.036	3.12	0.416	0.1	0.236
RM 7311	5	0.1	0.404	0.404	2.506	0.416	0.96	1.61	3.63	0.404

snpOS0024	5	1.138	0.891	1.11	0.019	0.977	0.076	0.076	0.746	0.285
snpOS0306	5	1.164	1.715	1.202	0.518	2.011	1.06	0.786	0.826	0.685
RM 527	6	0.9	0.025	0.628	2.506	2.506	1.231	1.225	0.628	0.225
HvSSR06-03	6	0.027	0.409	0.625	0	0.909	0.232	0.225	0.937	0.106
HvSSR06-54	6	0.025	0.225	0.025	0.902	0.1	0.226	0.4	0.628	2.088
HvSSR06-56	6	1.128	0.676	0.409	0.113	0.625	1.83	0.029	0.644	0.274
HvSSR06-75	6	0.613	0.114	1.6161	1.225	0.313	0.013	0.025	1.616	0
ILA-3	6	1.616	4.912 **	0.1	0.416	1.758	0.106	0.416	0.1	1.6
PR 62-3	6	1.556	0.326	0.338	1.342	0.389	0.127	0.242	0.529	0.283
HvSSR07-09	7	0.444	1.894	1.778	2.427	0.153	0.017	3.506	1.67	0.018
HvSSR07-44	7	0.106	0.113	0.533	1.025	3.636	3.956 ^+	1.025	0.11	0.404
HvSSR08-28	8	0.902	1.758	0.92	1.666	0.902	2.506	0.96	0	0.102
snpOS0022	8	0.509	1.21	0.823	1.178	1.297	1.803	1.008	2.482	0.293
PR 83-2	8	0.345	1.421	2.173	0.298	0.098	1.541	0.018	1.369	0.236
PR 096-4	9	0.145	0.662	0.316	0.114	0.625	0.632	0.444	0.542	5.253 **
HvSSR10-30	10	0.645	1.603	5.95 ^+	2.348	0.119	1.636	2.19	1.32	0.104
HvSSR10-34	10	1.231	1.244	5.657 **	2.506	0	1.616	0.625	2.55	1.231
HvSSR10-38	10	0.025	0.232	0	0.96	0.025	1.225	0.416	0	0.1
PR 101-1	10	3.6	0.902	0.404	0.404	2.558	2.506	0	1.667	0
HvSSR11-13	11	0.658	0	0.989	0.658	0.635	0.251	0.658	0.426	3.186
snpOS0015	11	1.111	1.053	1.108	0.944	1.111	1.078	0	0.765	1.1
snpOS0314	11	0.508	0	0	1.032	1.138	1.034	1.032	0	0.656
snpOS0315	11	0.378	0.921	0.406	0.336	0.762	0	0.057	0.121	0.036
PR 111-3	11	0.083	0.282	0.182	0.039	0.278	0.556	0.147	0.221	0.245
HvSSR12-34	12	2.633	1.666	1.429	2.666	2.506	3.6	0.416	1.544	0.902
HvSSR12-35	12	0.139	0.707	1.776	0.233	0.703	0.049	0.498	3.666	3.793
snpOS0006	12	0.894	0.004	0	0.99	0.894	0.536	0.045	0.788	0.199
snpOS0316	12	4.244^+	4.343 **	0.084	0.181	3.11	0.06	4.161 ^+	0.87	0.895
snpOS0317	12	0.706	0.023	0.098	0.509	0.4	0.066	0.121	0.232	0.046
snpOS0318	12	1.64	1.692	1.044	0.296	3.206	2.128	0.378	2.551	1.339
K 46-2	12	0.102	0.4	1.6	0.902	2.506	0.439	1.616	4.912 ^+	1.616

Table value (0.05)= 3.841; Values significant for both LR 23 and LR 26 type alleles & 23 and 26 alleles associated with good and bad genotypes, respectively is shown as **. Only LR 23 type allele= * and ^= only LR 26 type allele; superior allele=+; negative allele=-

4.10.2 Regression analysis results

Through regression analysis, 10 markers were found to be associated with yield attributing traits and are involved in imparting more than 5 % phenotypic variation for the trait associated with them (Table 4.17).

HvSSR02-10 is associated with TN 60 ($R^2=0.0548$), FG ($R^2=0.0901$), F % ($R^2=0.1271$), GYPP ($R^2=0.1214$), PUE at harvest ($R^2=0.0823$) and PUE at 60 DAT ($R^2=0.1044$). Marker HvSSR02-14 is associated with TN 30 ($R^2=0.0806$), TN 60 ($R^2=0.0531$), FG ($R^2=0.1105$), F % ($R^2=0.1611$), GYPP ($R^2=0.1349$), PUE at harvest ($R^2=0.0891$) and PUE at 60 DAT ($R^2=0.0845$). HvSSR02-33 is associated with FG ($R^2=0.103$) and GYPP ($R^2=0.0797$). Markers HvSSR10-30, 10-34 and PR1-2 are associated with PN ($R^2=0.0657$, 0.0943 and 0.0604), respectively. Candidate gene based markers PR03102 and 3103 are associated with F % ($R^2=0.0564$). PR9-2 is associated with TN 30 ($R^2=0.0532$), F % ($R^2=0.0597$), GYPP ($R^2=0.0776$) and PUE at 60 DAT ($R^2=0.1004$) while PR096-4 is associated with PUE at 60 DAT ($R^2=0.0593$), snpOS305 is associated with FG ($R^2=0.0718$), F % ($R^2=0.122$), GYPP ($R^2=0.120$) and PUE at harvest ($R^2=0.0712$). Marker snpOS316 is associated with TN 30 ($R^2=0.0604$) and TN 60 ($R^2=0.053$).

Five markers explained more than 10% of the phenotypic variation (Fig. 4.21).

Table 4.17 Regression values of each polymorphic marker with yield attributing traits

Marker Name	Chr #	TN 30	TN 60	PN	FG	100SW	F %	GYPP	PUE (harvest)	PUE (60 DAT)
HvSSR01-76	1	0.024	0.005	0.008	0.009	0.009	0.047	0.010	0.018	0.010
PR 15-5	1	0.011	0.009	0.006	0.000	0.008	0.006	0.000	0.000	0.001
snpOS0296	1	0.026	0.034	0.010	0.046	0.048	0.045	0.049	0.033	0.008
HvSSR02-01	2	0.000	0.000	0.000	0.002	0.000	0.001	0.002	0.001	0.000
PR 9-2	2	0.053	0.018	0.003	0.048	0.005	0.060	0.078	0.016	0.100
HvSSR02-10	2	0.041	0.055	0.026	0.090	0.002	0.127	0.121	0.082	0.075
HvSSR02-14	2	0.081	0.053	0.031	0.115	0.006	0.162	0.135	0.089	0.085
HvSSR02-17	2	0.022	0.004	0.000	0.013	0.033	0.046	0.018	0.002	0.014
HvSSR02-33	2	0.004	0.006	0.032	0.103	0.002	0.018	0.080	0.025	0.003
HvSSR02-44	2	0.006	0.001	0.005	0.001	0.002	0.011	0.000	0.006	0.022
HvSSR02-62	2	0.010	0.020	0.025	0.027	0.008	0.015	0.027	0.007	0.004
snpOS0298	2	0.000	0.009	0.003	0.034	0.049	0.040	0.047	0.009	0.027
snpOS0299	2	0.023	0.001	0.000	0.034	0.014	0.025	0.048	0.024	0.016
snpOS0302	2	0.004	0.015	0.000	0.001	0.000	0.002	0.002	0.000	0.004
PR 5-2	2	0.000	0.002	0.001	0.011	0.012	0.035	0.016	0.000	0.005
PR 10-1	3	0.002	0.005	0.000	0.001	0.003	0.001	0.000	0.000	0.002
PR03102	3	0.019	0.014	0.000	0.010	0.003	0.056	0.009	0.000	0.045
PR03103	3	0.019	0.014	0.000	0.010	0.003	0.056	0.009	0.000	0.045
FR33-3	3	0.014	0.031	0.023	0.047	0.004	0.029	0.042	0.016	0.000
PR1-2	3	0.011	0.027	0.060	0.029	0.000	0.000	0.027	0.047	0.000
snpOS0303	3	0.002	0.000	0.004	0.010	0.015	0.005	0.006	0.023	0.008
HvSSR04-49	4	0.001	0.007	0.000	0.007	0.034	0.031	0.001	0.000	0.017
snpOS0068	4	0.002	0.003	0.017	0.001	0.001	0.005	0.000	0.001	0.010
snpOS0304	4	0.001	0.000	0.001	0.001	0.017	0.002	0.002	0.006	0.004
snpOS0305	4	0.039	0.039	0.006	0.072	0.002	0.122	0.12	0.071	0.000
HvSSR05-59	5	0.004	0.020	0.030	0.013	0.022	0.037	0.020	0.039	0.017
RM 7311	5	0.005	0.001	0.008	0.024	0.021	0.019	0.011	0.030	0.006
snpOS0024	5	0.019	0.013	0.044	0.010	0.010	0.000	0.004	0.011	0.000
snpOS0306	5	0.020	0.006	0.000	0.000	0.002	0.018	0.026	0.007	0.000
RM 527	6	0.000	0.000	0.003	0.013	0.047	0.015	0.003	0.006	0.001
HvSSR06-03	6	0.000	0.017	0.001	0.002	0.007	0.016	0.001	0.005	0.009
HvSSR06-54	6	0.013	0.003	0.004	0.015	0.012	0.004	0.008	0.017	0.018
HvSSR06-56	6	0.030	0.046	0.034	0.014	0.006	0.010	0.012	0.028	0.020
HvSSR06-75	6	0.007	0.018	0.037	0.023	0.005	0.002	0.018	0.022	0.000
ILA-3	6	0.041	0.028	0.000	0.001	0.048	0.001	0.004	0.001	0.030
PR62-3	6	0.001	0.003	0.000	0.002	0.005	0.003	0.003	0.001	0.001
HvSSR07-09	7	0.020	0.045	0.036	0.034	0.000	0.000	0.023	0.008	0.026
HvSSR07-44	7	0.003	0.001	0.004	0.002	0.010	0.034	0.001	0.001	0.001
HvSSR08-28	8	0.012	0.016	0.012	0.040	0.002	0.029	0.042	0.008	0.012
snpOS0022	8	0.002	0.020	0.005	0.020	0.019	0.026	0.023	0.026	0.000
PR83-2	8	0.001	0.014	0.027	0.002	0.000	0.013	0.002	0.040	0.000
PR096-4	9	0.019	0.015	0.031	0.008	0.000	0.001	0.011	0.013	0.059
HvSSR10-30	10	0.006	0.029	0.066	0.045	0.004	0.043	0.050	0.028	0.000
HvSSR10-34	10	0.017	0.038	0.094	0.041	0.009	0.019	0.037	0.039	0.002
HvSSR10-38	10	0.000	0.002	0.009	0.010	0.000	0.003	0.008	0.007	0.006
PR101-1	10	0.016	0.006	0.001	0.000	0.003	0.014	0.001	0.013	0.001
HvSSR11-13	11	0.011	0.007	0.004	0.001	0.001	0.001	0.000	0.007	0.006
snpOS0015	11	0.040	0.010	0.004	0.003	0.020	0.004	0.000	0.010	0.001
snpOS0314	11	0.014	0.002	0.001	0.008	0.045	0.035	0.023	0.000	0.004
snpOS0315	11	0.038	0.040	0.005	0.025	0.011	0.034	0.008	0.002	0.026
PR111-3	11	0.000	0.001	0.005	0.001	0.046	0.005	0.000	0.002	0.012
HvSSR12-34	12	0.003	0.014	0.035	0.005	0.002	0.008	0.009	0.022	0.000
HvSSR12-35	12	0.004	0.010	0.001	0.008	0.010	0.001	0.001	0.044	0.011
snpOS0006	12	0.000	0.031	0.002	0.003	0.000	0.042	0.012	0.000	0.000
snpOS0316	12	0.060	0.053	0.011	0.006	0.007	0.028	0.039	0.000	0.004
snpOS0317	12	0.004	0.008	0.001	0.007	0.002	0.009	0.008	0.001	0.009
snpOS0318	12	0.006	0.005	0.005	0.001	0.046	0.013	0.000	0.006	0.003
K46-2	12	0.002	0.012	0.030	0.008	0.050	0.000	0.013	0.036	0.010

Marker showing more than 5 % phenotypic variation for the associated trait is marked in red.

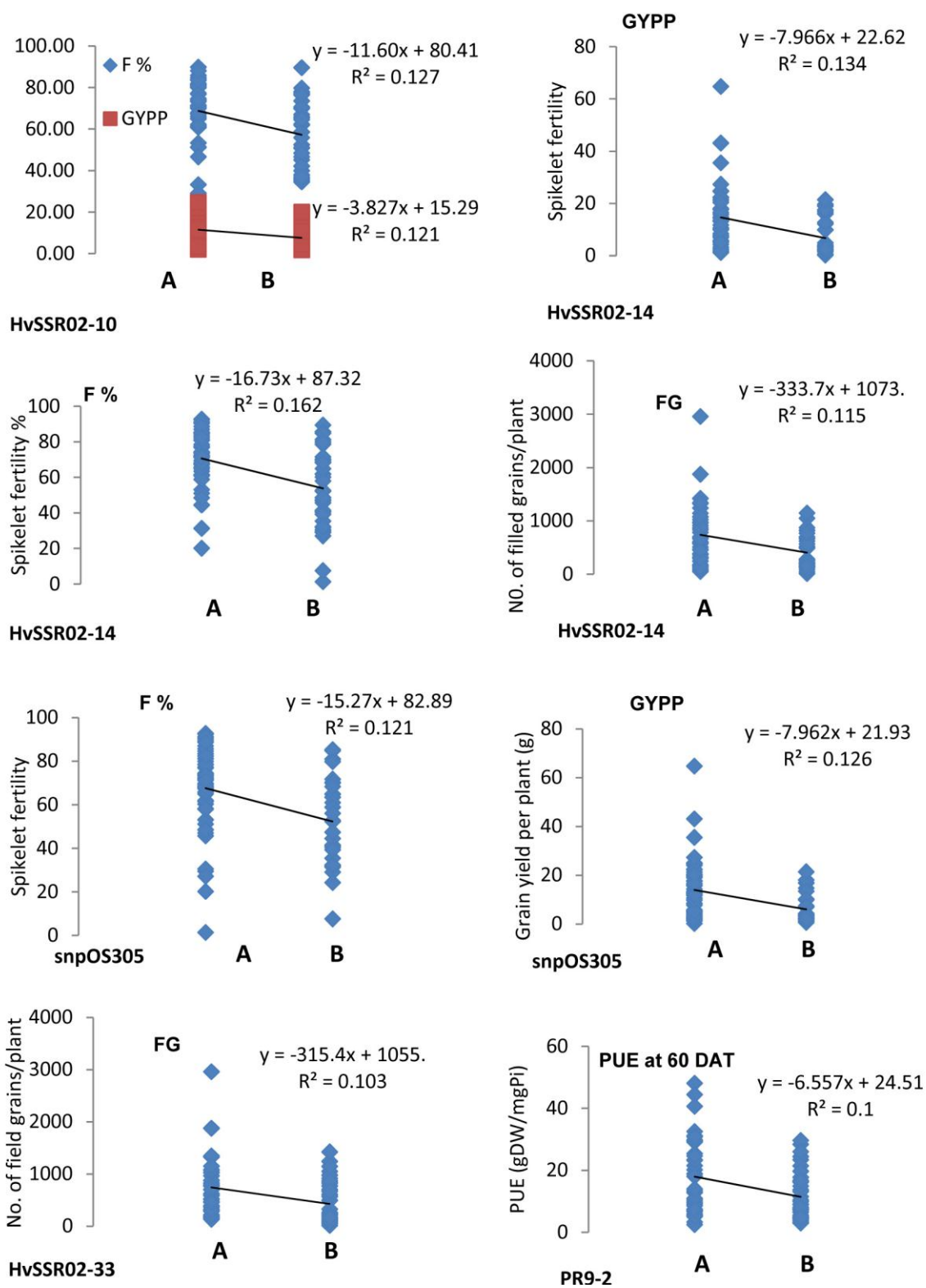


Fig.4.21 Regression graph of the five markers showing more than 10% phenotypic variation for the associated trait in selected 80 lines. A=LR 23 type allele and B= LR 26 type allele

4.10.3 STRUCTURE analysis results

To analyze the percentage ancestry of the selected genotypes, we performed a Bayesian clustering analysis using STRUCTURE with varying levels of K. Specifically, at K =2, we separated the two main groups, LR 23 type (shown in green) and LR 26 type (shown in red) (Fig.4.22).

While most of the selected progeny could be classified into one of the two groups at K= 2, we classified 32 genotypes as admixtures because they showed less than 80% of estimated ancestry derived from any single subpopulation (Table 4.18). When the proportion of ancestry required to identify subpopulation identity was reduced to 70%, only 20 genotypes could not be clustered clearly within a single sub-type and were classified as admixed. Total of 24 lines is clustered within a LR 23 type group (13 lines from good and 11 lines from bad performing group). Similarly, another 24 lines is clustered within a LR 26 type (8 lines from good and 16 lines from bad performing group) (Table 4.18).

The distribution of the two types *i.e.*, LR 23 and LR 26 type was uniform in the selected 80 genotypes as the overall proportion of membership of the sample in LR 23 type cluster and LR 26 type cluster was 0.497 and 0.503, respectively.

The proportion of LR 23 and LR 26 type was uniform in the superior lines (Fig. 4.21; Table 4.18).

Table 4.18. Proportion of parental ancestry of the 80 selected genotypes based on STRUCTURE version 2.3.2.1 at K=2

Sl no.	Genotypes	Ancestry proportion		Sl no.	Genotypes	Ancestry proportion	
1	LR23	0.058	0.942	1	LR23	0.058	0.942
2	LR26	0.947	0.053	2	LR26	0.947	0.053
3	230(3)-1	0.503	0.497	43	161(3)-1	0.08	0.92
4	154(2)-1	0.036	0.964	44	87(3)-4	0.565	0.435
5	198(2)-1	0.963	0.037	45	120(4)-2	0.708	0.292
6	195(5)-3	0.284	0.716	46	29(1)-3	0.935	0.065
7	132(2)-1	0.02	0.98	47	60(3)-4	0.934	0.066
8	61(1)-2	0.106	0.894	48	90(1)-2	0.955	0.045
9	18(2)-1	0.146	0.854	49	62(4)-4	0.967	0.033
10	80(1)-1	0.817	0.183	50	156(4)-2	0.039	0.961
11	95(4)-1	0.167	0.833	51	37(4)-5	0.101	0.899
12	90(5)-5	0.785	0.215	52	49(1)-3	0.957	0.043
13	122(5)-1	0.932	0.068	53	51(4)-3	0.942	0.058
14	73(4)-1	0.897	0.103	54	23(3)-3	0.647	0.353
15	8(4)-4	0.043	0.957	55	48(3)-2	0.968	0.032
16	153(4)-5	0.269	0.731	56	168(4)-3	0.976	0.024
17	206(5)-5	0.253	0.747	57	55(2)-3	0.42	0.58
18	220(2)-4	0.916	0.084	58	7(3)-1	0.043	0.957
19	181(4)-2	0.444	0.556	59	235(1)-2	0.968	0.032
20	201(2)-2	0.56	0.44	60	205(4)-2	0.612	0.388
21	83(4)-2	0.427	0.573	61	36(4)-3	0.074	0.926
22	87(4)-2	0.453	0.547	62	196(4)-1	0.531	0.469
23	222(3)-5	0.395	0.605	63	19(4)-1	0.038	0.962
24	33(1)-1	0.954	0.046	64	50(4)-1	0.375	0.625
25	188(4)-5	0.259	0.741	65	117(5)-1	0.109	0.891
26	102(4)-4	0.035	0.965	66	41(5)-5	0.841	0.159
27	17(3)-4	0.942	0.058	67	224(1)-3	0.071	0.929
28	11(4)-1	0.04	0.96	68	180(2)-1	0.627	0.373
29	34(3)-4	0.077	0.923	69	171(2)-1	0.83	0.17
30	152(5)-1	0.148	0.852	70	42(3)-2	0.055	0.945
31	122(5)-5	0.504	0.496	71	196(5)-1	0.612	0.388
32	215(2)-4	0.629	0.371	72	2(4)-3	0.061	0.939
33	229(4)-3	0.606	0.394	73	165(2)-4	0.961	0.039
34	172(3)-3	0.177	0.823	74	93(1)-2	0.575	0.425
35	59(4)-4	0.494	0.506	75	124(1)-1	0.27	0.73
36	22(2)-4	0.088	0.912	76	123(1)-2	0.516	0.484
37	163(1)-5	0.217	0.783	77	221(1)-5	0.929	0.071
38	114(2)-4	0.074	0.926	78	78(1)-1	0.751	0.249
39	81(4)-3	0.432	0.568	79	170(1)-5	0.891	0.109
40	193(1)-1	0.286	0.714	80	133(1)-1	0.978	0.022
41	34(3)-1	0.893	0.107	81	192(4)-4	0.187	0.813
42	65(3)-5	0.53	0.47	82	46(3)-2	0.818	0.182

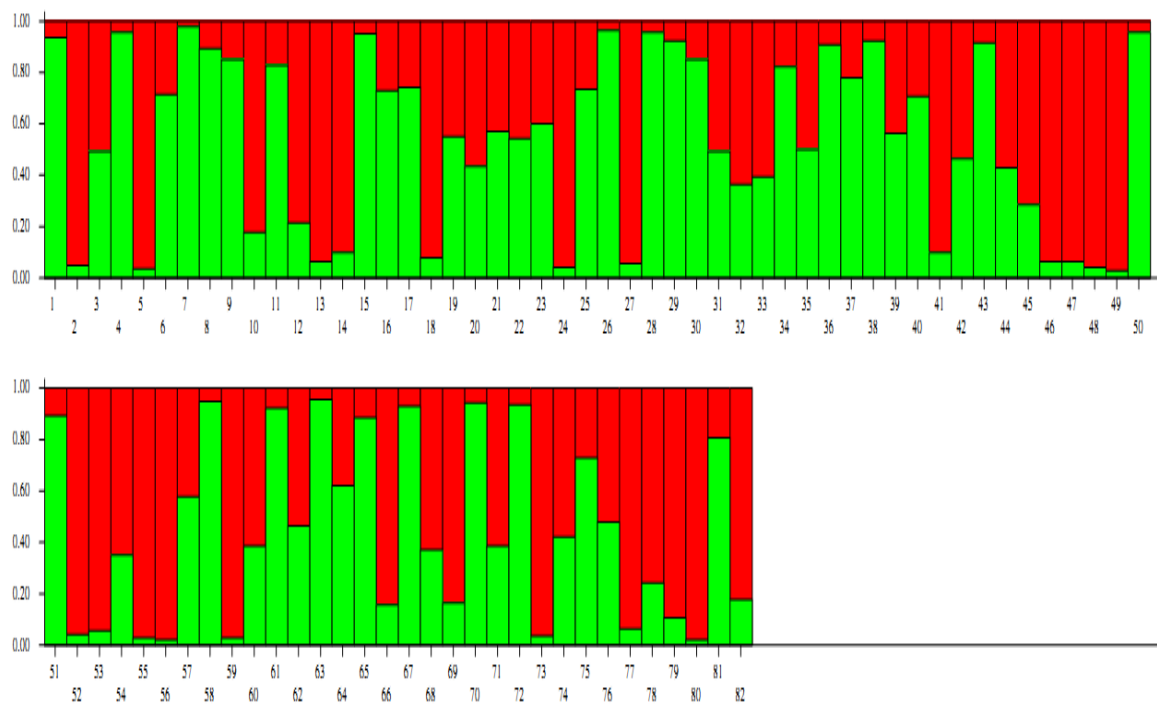


Fig. 4.22 STRUCTURE output showing distribution of parental ancestry in selected 40 good and 40 bad performing lines. Red and green colors represent LR 26 and LR 23 type parental ancestry, respectively. Numbers 1-82 represent the samples as listed in Table 4.18.

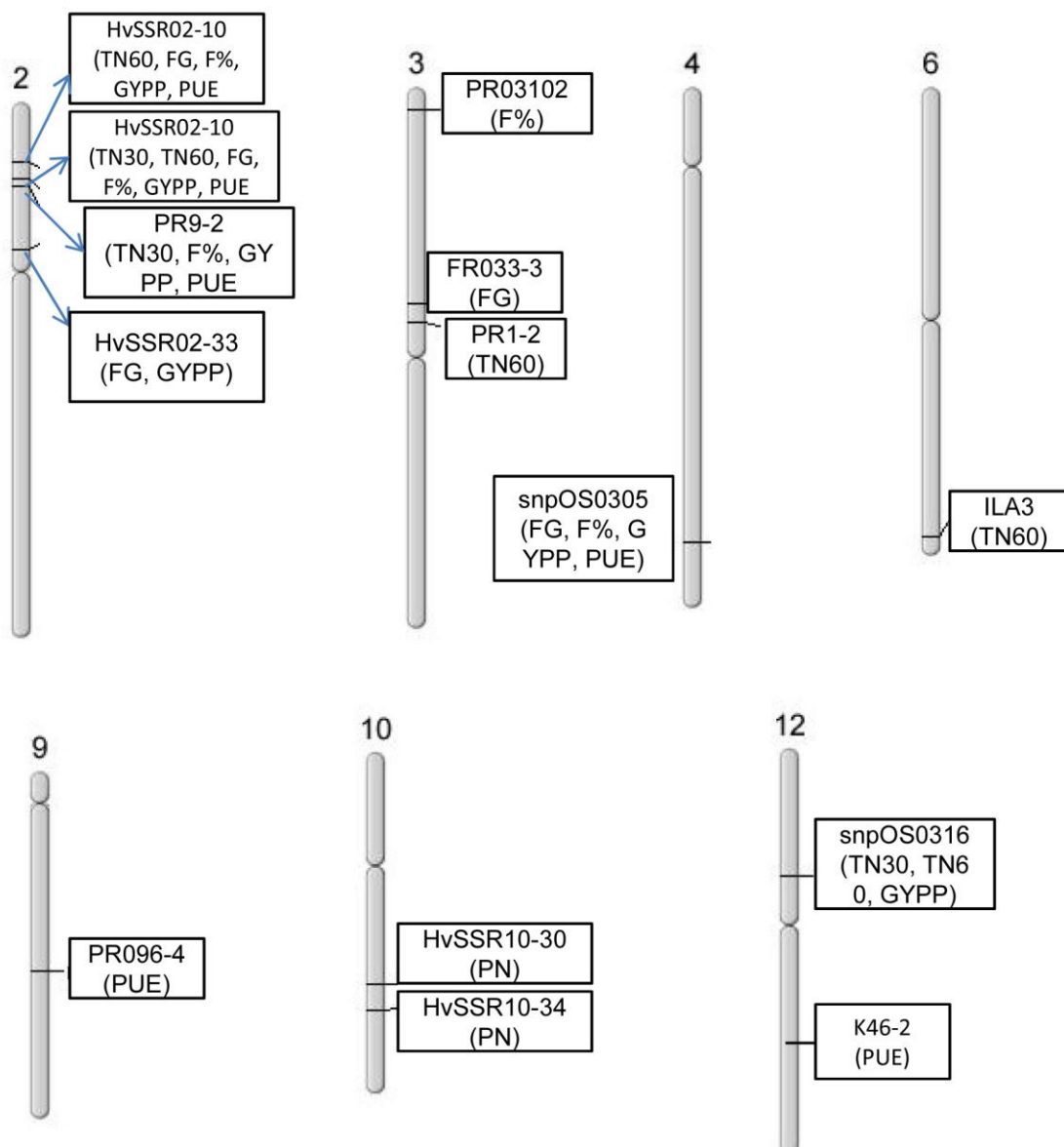


Fig. 4.23 Chromosomal map showing position of markers that are significantly associated with yield attributing traits

4.10.4 Genotyping the RIL populations with significant markers

Markers that are found to be significantly associated with yield attributing traits (Fig. 4.23) were used for genotyping the total population. However, regression analysis showed only one marker snpOS0305 contributing more than 5% phenotypic variation for a trait *i.e.* spikelet fertility percentage with regression (R^2) value of 0.0559.

Chapter 5

Discussion

Across world, million hectares of land is affected by soil acidity and making it unsuitable for crop cultivation. This problem is the predominant problem affecting agriculture in northeastern part of India. Phosphorus in acidic soil becomes unavailable to the crop plants due to its reaction with cations like aluminum and iron and is one of the main reasons for lower productivity of crops like rice in this region. Identifying and introgressing genes imparting P deficiency tolerance into the crop varieties is a sustainable, effective and efficient way to improve the productivity in this region.

5.1 Identification of *OsGLK* members with a potential role in low P tolerance

In silico analysis of *OsGLK*, it is observed that 8 GLK genes namely; *K6*, *K14*, *K15*, *K23*, *K31*, *K32*, *K36* and *K40* have biological function as phosphate starvation response regulator. Seventeen members of *OsGLK* gene family carry copies of P1BS *cis*-acting elements and 3 genes (*K15*, *K31* and *K40*) carrying P1BS element has annotated biological function as phosphorus starvation response regulator.

P1BS is a *PHR1*-binding sequence found in the upstream regions of phosphate starvation responsive genes (Rubio *et al.*, 2001; Schünmann *et al.*, 2004). *GLK* genes carrying multiple copies of *cis* acting elements like P1BS suggests that they be regulated by levels of Pi. Two *OsPHR* genes designated as *OsPHR1* and *OsPHR2* play role in Pi starvation signaling pathway by regulating the expression of Pi-starvation induced genes (Zhou *et al.*, 2008). *OsPHR1* is *OsGLK15* and in rice, *PHR1* is negatively regulated in a Pi-dependent manner by proteins containing *SPX* domain (Wang *et al.*, 2014; Puga *et al.*, 2014; Wild *et al.*, 2016).

OsGLK31 is *OsPHR4* and *OsPHR4* has been reported to be induced by Pi starvation. Over expression *OsPHR4* resulted in higher accumulation of Pi in the shoots. The expression of *OsPHR4* is positively regulated by *OsPHR1*, *OsPHR2* and *OsPHR3*. *OsPHR4* regulates the induction of downstream Pi-starvation genes. Transgenic lines with silenced *OsPHR4* gene showed growth retardation and repression of Pi starvation inducible genes (Ruan *et al.*, 2017).

OsHKT1;1 in rice is *OsGLK40*. *OsGLK40* has biological role of phosphorus starvation response regulator. *OsHKT1;1* was reported to playing role in reducing Na⁺ accumulation in shoots to cope with salt stress and was expressed mainly in the phloem of leaf blades (Wang *et al.*, 2015). The *oshkt1;1* mutant plants were found to be hypersensitive to salt stress.

In our study, real time PCR/qPCR showed that ten *OsGLK* genes namely *K4*, *K7*, *K8*, *K10*, *K14*, *K15*, *K19*, *K30*, *K37* and *K41* are differentially expressed under low P condition. Other than *K15* (*PHR1*), nine *GLK* genes were identified to be differentially regulated by Pi levels through qPCR in this study for the first time. These genes will form the basis of future studies on low P tolerance in acidic soils.

Among the ten genes that showed differential expression under P deficiency condition, six members namely; *K4*, *K7*, *K10*, *K15*, *K30* and *K41* carry P1BS cis acting elements suggesting that their expression might be regulated by binding to this already reported region in 5' UTR. The sequence of the upstream regions of these genes will be of interest especially in rice genotypes showing contrasting response to low P levels. Infact, gene *K10* is up regulated in tolerant genotypes and down regulated in the susceptible genotypes evaluated after 24 hour stress treatment. The expression of *K30* is down regulated in one tolerant genotype whereas it is up regulated in one susceptible genotype. The 5' UTR sequence of these two genes across tolerant and susceptible genotypes might shed some light into possible differences for differential gene expression. Furthermore, the four members (*K8*, *K14*, *K19* and *K37*), which lack P1BS show interesting expression pattern and their 5' UTR could be explored further. Genes *K14*, *K19* and *K37* were differentially regulated in LR 23 and BAM 350, whereas gene *K8* was differentially regulated in LR 26 and BAM 811. Maybe the gene expression induction is influenced by other factors and not just levels of P. Furthermore, root expression was not checked for these genes as they have been basically implicated in shoot related functions, but this could be part of future studies. Role of lncRNAs (long non-coding RNAs) (Bazin and Bailey-Serres, 2015) as well as regulation by histone acetyl transferase (Jia *et al.*, 2015) is now clearly established in the modulation of root system architecture and in maintaining the phosphate homeostasis. It has been shown that histone acetyl transferase epigenetically regulates all the major transcription factors (*PLT1*, *PLT2*, *SCR*, *SHR*, *WOX5*) involved in maintaining root stem cells and the quiescent centre (QC) in Arabidopsis (Jia *et al.*, 2015). Role of ribo-regulator (lncRNAs) along with miR399 (induced by Pi starvation) in regulating *PHO2* is well documented across species like rice, rice, Arabidopsis and Medicago (Liu *et al.*, 2009; Bazin and Bailey-Serres, 2015)

5.2 Identification of rice chromosome 2 as potential regulon for better performance under lowland acidic soils tolerance

Yield is a complex quantitative trait. In rice, 8,646 QTLs are reported till date (Gramene database). This includes 22 QTLs for filled grain number, 398 for grain yield, 93 for grain yield per panicle, 80 for grain yield per plant and 239 for panicle number.

Marker trait association in the RIL population screened in lowland acidic soil led to the identification of chromosome #2 (region from 4.29 Mb till 5.92 Mb) as one of the key chromosome for imparting better performance under low land acidic soil. This region is flanked by two markers HvSSR020-10 (RM12570) at position 4.29 Mb and PR9-2 underlying WD40-38 gene (02g11060) lies at position 5.92 Mb (Fig 5.1). Both the markers are genic markers, with HvSSR020-10 lying within a calmodulin binding protein (02g08120) and PR9-2 underlying within WD40-38 gene (02g11060). Calmodulin is a key calcium binding protein playing a role in signal transduction with a known role in regulation of ion transporters. The Arabidopsis ortholog of 02g08120, AT2G18750, is highly expressed in stems (Reddy *et al.*, 2002). It has calcium binding property and it interacts with AtCaM15 which is localized in vacuoles (Yamaguchi *et al.*, 2005). The interaction of AtCaM15 with AtNHX1 is calcium and pH dependent and AtCaM15 modified the ion selectivity of AtNHX1. WD40-38 is a G-beta repeat domain containing protein already reported to be differentially expressed only in LR 23 under low P conditions (Yumnam *et al.*, 2017).

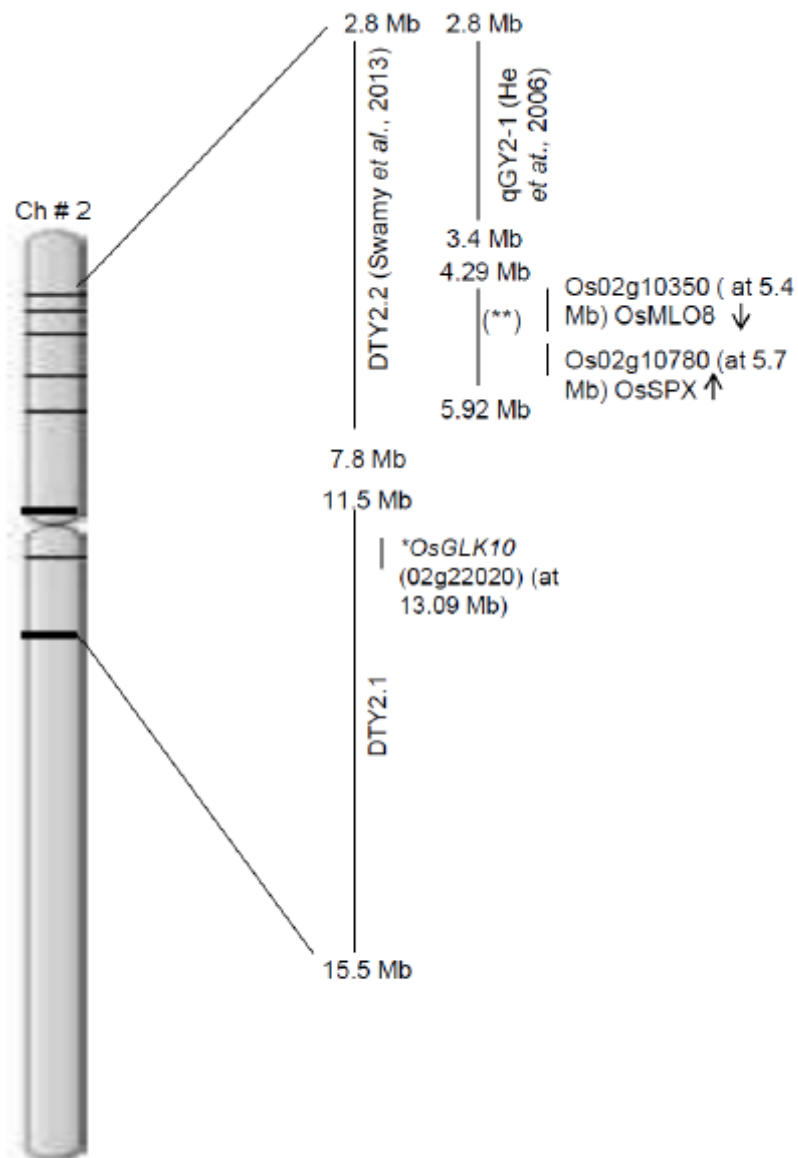
This 1.63 Mb region overlaps with one of the large effect QTLs, DTY 2.2 reported previously in IR 64 background (Swamy *et al.*, 2013) (Fig 5.1). Another QTL *i.e.* qGY2-1 flanked by markers RM 7652 and RM 6842 also lies at position 2.8-3.4 Mb on chromosome 2 (He *et al.*, 2006).

This region does not contain any of the PSTOL1 putative downstream genes as reported previously by Gamuyao *et al.*, 2012. Previously, a QTL for PUE was identified on chromosome #2 (Wissuwa *et al.*, 1998). This QTL explained 9.8 % of the variation where the positive allele was derived from susceptible genotype, Nipponbare. Furthermore, the effect of substituting Nipponbare allele with Kasalath allele (tolerant genotype) was negative. This region is flanked by RFLP markers C365 (6.58 Mb) and G277 (7.53 Mb).

Apart from calmodulin and WD40 genes, this region also contains genes like OsMLO8 (02g10350) which is down regulated in Sahbhagi Dhan (LR 23)

under low P treatment condition (Tyagi and Rai, 2017). MLO domain proteins are the only major higher plant family to possess 7 trans membrane domains. It has been suggested that these protein functions as G-protein coupled receptors in plants. *OsSPX* (SPX domain containing protein 2) (02g107080) also lies on this region. *OsSPX 2* is reported to be up regulated in LR 23 and LR 26 (tolerant genotypes) (Tyagi and Rai, 2017). In rice *SPX1* and *SPX2* inhibit phosphate starvation responses by interacting with *PHR2* in a phosphate dependent manner (Wang et al., 2014b). These proteins interact with *PHR2* on their *SPX* binding domain, inhibiting its binding to P1BS. The qPCR data, revealed *K10* which lies on ch#2 at position 13.09 Mb (Fig. 5.1), as the only GLK member differentially regulated in tolerant and susceptible genotypes in response to P deficiency conditions in shoots. This gene in turn lies within the fine mapped region of DTY2.1.

This data suggest that this region on chromosome 2 acts as a major regulon for modulating better performance in response to low P conditions in poor soils. The genes and genotypes harbouring the genes would form basis of future studies on low P tolerance in rice under acidic soils. The markers identified in the current study, after further validation could be used for marker assisted selection for higher yield under lowland acidic conditions.



** Region flanked by marker HvSSR(RM12570) and PR 9-2 02-10 identified in current experiment playing role in better performance under low P condition

* *OsGLK10* identified in current study to be differentially expressed in tolerant and susceptible genotypes under low P condition.

↓ *OsMLO8* gene reported to be downregulated in LR 23 (Tyagi and Rai, 2017)

↑ *OsSPX* gene up regulated in tolerant genotype (Tyagi and Rai)

Fig. 5.1. chromosomal map showing position of major QTLs on ch#2.

Chapter 6

Summary and Conclusion

Nutrient deficiency is one of the major abiotic factors that affects the production and productivity of rice and plants as a whole. The present study was conducted to characterize a transcription factor family (G2 like or GLK) using *in silico* and wet lab approach to look into its role in P deficiency tolerance. This study also used marker trait association to identify alleles, genes and genotypes for better performance under low P acidic soils in a bi-parental mapping population (F₆) derived from two P deficiency tolerant genotypes ((Sahbhagi Dhan (SD) and Chakhao Poreiton (CP)). The results of the present study are summarized below:

- *In silico* results show that *OsGLKs*, a MYB transcription factor family belongs to super family GARP. It has 62 members in *Oryza sativa* japonica type and 52 members in indica type including splice variants.
- Its main molecular function is sequence specific DNA binding and have varied biological functions namely transcription factor for chloroplast development, transcription factor for phosphate starvation response, transcription factor for the circadian clock oscillation.
- There are hundreds of cis-acting elements present in 5' UTR region of *OsGLK* genes in multiple copies among which 17 members were found to be carrying multiple copies of P1BS elements which has important role in phosphate starvation response regulation.
- For three members of *OsGLK*, information regarding gene disruption phenotype is available along with the mutant id. Information about *OsGLK* orthologs in maize was also assembled.
- Phylogenetic analysis revealed many sub clusters for *OsGLK* family members on the basis of presence and combination of 5 major protein domain namely a) coil type b) MYB homeodomain-like c) signal transduction response regulator domain d) MYB CC-type transcription factor domain and e) short chain dehydrogenase/reductase domain.
- Semi quantitative PCR was conducted on cDNA synthesised from total RNA that was extracted from shoots of 24 hours low P treated and control LR 23

and LR 26 plants at seedling stage. Through semi quantitative PCR, two genes (*K14* and *K37*) were identified to be differentially expressing under low P condition. *K14* is found to be up-regulated in LR 23 and *K37* is found to be down regulated in LR 26.

- From available root transcriptome data, four genes namely *K8*, *K10*, *K19* and *K37* were identified to be differentially expressed in roots growing under low P conditions for 15 days. Differentially expressing genes that were identified in semi quantitative PCR and from root transcriptome data namely *K8*, *K10*, *K14*, *K19* and *K37* and *OsGLK* genes carrying multiple copies of P1BS cis acting elements (*K4*, *K7*, *K15*, *K30*, *31*, *K41* and *51*) were subjected to real time PCR. Real time PCR was performed on cDNA of four genotypes comprising of two tolerant (LR 23 and LR 26) and two susceptible (BAM 350 and BAM 811) genotypes. Through real time/qPCR, ten *OsGLK* genes (*K4*, *K7*, *K8*, *K10*, *K14*, *K15*, *K19*, *K30*, *K37* and *K41*) were identified to be differentially expressed under P normal and deficit conditions. Genes *K4*, *K7* and *K41* were found to be differentially expressed in susceptible genotypes. *K15* was differentially expressed in tolerant genotypes and *K8*, *K10*, *K14*, *K19*, *K30* and *K37* were differentially expressed in both susceptible and tolerant genotypes.
- RILs derived from two P deficiency tolerant genotypes (LR 23 and LR 26) are used to study marker trait association. On the basis of five major yield related traits (tiller number at 30 and 60 days after transplanting, filled grains per plant, spikelet fertility %, grain yield per plant and 100 seed weight) that show significant correlation with each other and with the yield, forty better performing lines and forty bad performing lines are selected from total population of 357 F₆ RILs. Four hundred sixteen (416) candidate gene based markers including markers from *OsGLK*, 350 SSR (including genic SSR) and 34 SNP that were distributed on all 12 chromosome were used for polymorphism survey between the two parents. Thirty one candidate gene based markers belonging to fourteen genes including *GLK* and *PSTOL1* gene, twenty five SSR marker including four genic SSR markers and eighteen SNPs were identified as polymorphic.
- A total of 57 markers comprising of 14 candidate gene based markers (one from each gene) 25 SSR markers (including genic SSR) and 18 SNP were used for genotyping the panel of 80 genotypes.

- Statistical analysis namely chi square test and regression test were conducted to confirm the association of markers with major traits of the rice plant under low P acidic soil condition. Marker trait association studies revealed that 14 markers viz., PR9-2, PR1-2, ILA3, PR03102 (*OsGLK51*), PR096-4 (*OsGLK41*), FR033-3, HvSSR02-10, HvSSR02-14, HvSSR02-33, HvSSR10-30, HvSSR10-34, snpOS0305, snpOS0316 and K46-2 to be significantly associated with yield attributing traits under lowland acidic field conditions. Two members of *OsGLK* family viz., *OsGLK41* (09g23200) and *OsGLK51* (03g03760) were found to be significantly associated with yield attributing traits like PUE 60 days ($\chi^2=5.253$, $R^2=0.0593$), spikelet fertility % ($R^2=0.0513$), respectively. Marker trait analyses lead to identification of chr. #2 as one of the key chromosome for imparting better performance under low land acidic soil. A set of 12 polymorphic markers lead to identification of nearly 6 Mb region (4.29-10.28 Mb) which was further reduced to 1.63 Mb region flanked by marker HvSSR02-10 and PR9-2 for better performance in terms of the traits like filled grains per plant, spikelet fertility %, grain yield per plant and phosphorus use efficiency at 60 days after transplanting.
- Parental ancestry shared by each of the selected 80 genotypes was calculated with the help of software called STRUCTURE version 2.3.2.1. Thirty two genotypes are classified as admixtures because they showed less than 80% of estimated ancestry derived from any single subpopulation. Total of 24 lines is clustered within a LR 23 type group (13 lines from good and 11 lines from bad performing group) and another 24 lines is clustered within a LR 26 type (8 lines from good and 16 lines from bad performing group). The distribution of the two types i.e., LR 23 and LR 26 type was uniform in the selected 80 genotypes as the overall proportion of membership of the sample in LR 23 type cluster and LR 26 type cluster was 0.497 and 0.503, respectively.

In conclusion, the results of this study suggest that, *OsGLK* transcription factor family has a greater role in P deficiency tolerance mechanism in rice plant. This conclusion is supported by *in silico* and wet lab findings as a) many of the GLK members are already reported to be involved in phosphate starvation signaling (*K6*, *K15*, *K32* and *K39*), b) presence of P1BS *cis*-acting elements in 17 of its member and c) differential expression of *OsGLK* genes under low P condition in control and treated rice plants. Genes like *Os02g08120*, *Os02g11060*, *OsGLK41*, *OsGLK51*, *Os06g50920*, *Os10g31040* and genes linked to SNP markers snpOS0316 and snpOs0305 contribute towards better performance in terms of filled grains per

panicle, spikelet fertility %, grain yield per plant and PUE at harvest under acidic soils. These genes would form basis of future studies on low P tolerance in rice under acidic soils. The markers identified in the current study, after further validation could be used for marker assisted selection for higher yield under lowland acidic conditions.

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Annexure I

Cis acting elements present in 5' UTR from CDS

Cis-acting elements

(+)=Positive strand (-)=Negative strand

Name of Primer	Gene	TATABOX	CAATBOX	GATABOX	GT1 CONSENSUS	MYB1AT	ERELEE4	MYC CONSENSUSAT	GT1GMSCA M4	CBFHV	GCCCORE	P1BS	IBOXCORE	INRNTPSA DB	LTRECOR EATCOR1 5
CAU-G2-RT24	OsGLK1	3 copies at 127(-), 284(+) 320(+)	10 copies at 19 (-), 94(-), 136(-), 148(-), 164(-), 292(+), 362(-), 557(-), 856(+), 987(-)	9 copies at position 57(+), 78 (+), 161(+), 206(-), 212(+), 409(-), 448(-), 614(-), 686(+)	5 copies at 57(+), 392(+), 637(+), 801(+), 826(+)	1 copy at 231(-)	1 copy at 11(-)	4 copies at 376(±) 884(±)	3 copies at 637(+), 802(+), 826 (+)	2 copies at 946(±)	NIL	NIL	2 copies at 57(+), 445 (-)	NIL	NIL
CAU-G2-RT02	OsGLK2	2 copies at 11(-), 84(-)	12 copies at 21(- , 28(-), 68(+), 117(-), 197(+), 225(+), 271(+), 390(-), 794(-), 809(+), 859(+), 884(-)	5 copies at 173(+), 383(-), 704(-), 847(+), 853(-)	3 copies at 121(+), 217(-), 288(+)	NIL	1 copy at 257(+)	10 copies at 374(±), 434(±), 445(±), 753(±), 776(±)	NIL	3 copies at 476(-), 584(±)	NIL	2 copies at 775(±)	NIL	3 copies at 807(+), 857(+), 882(-)	1 copy at 476(-)
CAU-G2-RT03	OsGLK3	1 copy at 800(+)	11 copies at 73(+), 86(-), 221(+), 244(+), 306(+), 308(-), 347(-), 506(+), 663(+), 665(-), 715(-)	5 copies at 27(+), 54(-), 456(-), 487(-), 653(+)	1 copy at 52(-)	1 copy at 168(-)	1 copy at 245(+)	16 copies at 10(±), 106(±), 270(±), 283(±), 306(±), 315(±), 402(±), 663(±)	NIL	3 copies at 466(+), 756(±)	3 copies at 607(-), 723(+), 953(-)	2 copies at 652(±)	3 copies at 27(+), 53(-), 455(-)	NIL	2 copies at 467(+), 840(+)
CAU-G2-RT04	OsGLK4	7 copies at 48(-), 72(+), 161(+), 414(-) 652(-), 660(- , 674(+)	10 copies at 91(+), 93(-), 175(-), 343(+), 397(+), 466(-), 470(+), 474(-), 733(-), 811(+)	6 copies at 45(+), 77(-), 111(+), 291(-), 357(+), 819(-)	10 copies at 75(-), 103(-), 111(+), 213(-), 220(-), 509(-), 611(-), 676(-), 817(-), 857(-)	3 copies at 419(+), 536(+), 668(+)	NIL	6 copies at 91(±), 182(±), 579(±)	3 copies at 103(-), 213(- , 509(-)	2 copies at 763(-), 905(+)	NIL	2 copies at 288(±)	3 copies at 76(-), 111(+), 818(-)	2 copies at 602(+), 793(-)	1 copy at 906(+)
CAU-G2-RT05	OsGLK5	3 copies at 200(+), 238(+), 299(+)	6 copies at 188 (-), 398(+), 403(+), 433(+), 440(+), 979(-)	10 copies at 135(+), 140(+), 155(+), 210(+), 268(+), 353(-), 505(-), 524(-), 646(-), 814(-)	4 copies at 435(-), 519(+), 735(-), 987(+)	1 copy at 953(-)	1 copy at 44(+)	6 copies at 130(±), 175(±), 663(±)	NIL	NIL	NIL	NIL	NIL	2 copies at 315(-), 896(-)	1 copy at 492(-)
CAU-G2-RT06	OsGLK6	1 copy at 799(+)	14 copies at 70(- , 203(+), 215(-), 251(+), 312(+), 327(-), 416(+), 449(-), 461(+), 592(+), 618(+), 716(+), 857(+), 963(-)	7 copies at 157(-), 180(-) 189(+), 302(+), 335(+), 579(-), 589(-)	5 copies at 155(-), 315(-), 577(-), 789(+), 978(+)	1 copy at 151(-)	NIL	12 copies at 89(±), 518(±), 582(±), 666(±), 849(±), 927(±)	2 copies at 315(-), 789(+)	1 copy at 206(+)	NIL	NIL	4 copies at 156(-), 179 (-), 302(+), 578(-)	5 copies at 213(-), 249(+), 310(+), 377(+), 679(+)	NIL

CAU-G2-RT07	OsGLK7	9 copies at 29(+), 121(-), 340(+), 358(+), 387(+), 494(+), 505(+), 518(+), 590(+)	9 copies at 150 (-), 204(+), 213(+), 258(+), 294(+), 551(+), 695(+), 957(-), 986(-)	13 copies at 43(+), 79(-), 159(+), 233(+), 281(+), 351(+), 535(+), 652(-), 657(+), 681(-), 775(-), 801(+), 944(-)	12 copies at 43(+), 77(-), 159(+), 233(+), 281(+), 410(-), 498(-), 510(-), 576(-), 595(-), 606(-), 773(-)	3 copies at 83(+), 237(+), 539(+)	NIL	10 copies at 451(±), 625(±), 810(±), 904(±), 920(±)	6 copies at 410(-), 498 (-), 510(-), 576(-), 595 (-), 606(-)	NIL	NIL	2 copies at 618(±)	7 copies at 43(+), 78(-), 159(+), 233(+), 281(+), 351(+), 774(-)	1 copy at 984(-)	1 copy at 21(-)
CAU-G2-RT08	OsGLK8	4 copies at 269(+), 289(-), 299(-), 309(+)	3 copies at 173 (-), 394(+), 800(+)	9 copies at 123(-), 129(+), 160(-), 166(+), 257(-), 370(+), 495(+), 504(-), 737(+)	2 copies at 255(-), 481(-)	2 copies at 352(+), 400(+)	NIL	4 copies at 695(±), 718(±)	1 copy at 481(-)	3 copies at 26(+), 466(-), 820(+)	3 copies at 938(-), 974 (-), 988(-)	NIL	2 copies at 166(+), 256(-)	1 copy at 650(-)	2 copies at 27(+), 466(-)
CAU-G2-RT10	OsGLK10	3 copies at 23(-), 701(+), 768(+)	5 copies at 78(-), 287(-), 401(+), 580(+), 716(+)	5 copies at 72(+), 272(-), 341(+), 348(+), 577(-)	7 copies at 21(+), 72(+), 120(+), 348(+), 367(-), 703(-), 770(-)	NIL	NIL	8 copies at 67(±), 217(±), 493(±), 627(±)	1 copy at 367(-)	NIL	1 copy at 977(-)	4 copies at 241(±), 671(±)	4 copies at 72(+), 271(-), 348(+), 574(-)	1 copy at 761(+)	1 copy at 837(+)
CAU-G2-RT14	OsGLK14	2 copies at 425(-), 544 (-)	14 copies at 83(+), 168(-), 209(+), 223(-), 248(+), 286(+), 420(+), 461(+), 485(+), 538(-), 623(-), 873(-)	11 copies at 80(-), 147(+), 199(-), 217(-), 594(+), 599(-), 706(-), 819(+), 959(+), 961(-), 986(+)	6 copies at 52(-), 78(-), 594(+), 799(-), 819(+), 951(+)	2 copies at 365(+), 934(-)	NIL	10 copies at 102(±), 466(±), 609(±), 671(±), 750(±)	2 copies at 52(-), 951(+)	NIL	NIL	NIL	3 copies at 79(-), 594(+), 819(+)	5 copies at 40(-), 277(+), 401(+), 459(+), 887(-)	NIL
CAU-G2-RT15	OsGLK15	4 copies at 272(+), 752(+), 790(+), 807 (-)	7 copies at 179 (-), 460(+), 553(+), 567(-), 584(-), 779(+), 946(-)	5 copies at 160(+), 216(+), 282(+), 390(-), 518(-)	6 copies at 263(+), 289(+), 383(+), 409(+), 507(-), 792(+)	2 copies at 135(-), 174(+)	NIL	10 copies at 235(±), 307(±), 364(±), 682(±), 974(±)	2 copies at 383(+), 409(+)	NIL	NIL	2 copies at 159(±)	NIL	1 copy at 830(+)	NIL
CAU-G2-RT16	OsGLK16	2 copies at 217(-), 225(-)	11 copies at 200(+), 214(+), 255(+), 272(+), 346(-), 513(-), 613(+), 625(-), 663(-), 672(+), 703(-), 800(-)	2 copies at 1(+), 895(-)	4 copies at 88(+), 319(+), 648(-), 690(+)	2 copies at 232(-), 287(-)	NIL	10 copies at 13(±), 165(±), 472(±), 712(±), 936(±)	2 copies at 88(+), 690(+)	1 copy at 487(-)	NIL	NIL	2 copies at 1(+), 894(-)	1 copy at 253(+)	1 copy at 487(-)
CAU-G2-RT17	OsGLK17	4 copies at 121(-), 158(-), 499(+), 537(+)	4 copies at 291(-), 416(-), 580(-), 900(-)	7 copies at 47(-), 150(-), 288(+), 299(+), 346(+), 560(+), 833(+)	6 copies at 45(-), 118(+), 281(+), 299(+), 339(+), 476(+)	NIL	1 copy at 363(+)	NIL	1 copy at 476(+)	1 copy at 627(+)	NIL	2 copies at 550(±)	3 copies at 46(-), 147(-), 299(+)	1 copy at 222(-)	1 copy at 516(+)
CAU-G2-RT18	OsGLK18	NIL	8 copies at 71(+), 90(-), 170(+), 189(-), 329(+), 838(+), 931(-), 938(+)	2 copies at 136(-), 856(-)	4 copies at 293(-), 590(-), 628(-), 730(-)	1 copy at 506(+)	NIL	2 copies at 394(±)	2 copies at 293(-), 590 (-)	NIL	NIL	NIL	NIL	2 copies at 10(-), 618 (+)	NIL

CAU-G2-RT19	OsGLK19	5 copies at 249(+), 310(-), 318(-), 533(+), 541(+)	9 copies at 72(-), 103(-), 120(-), 210(+), 345(+), 388(-), 436(-), 451(+), 457(-)	7 copies at 63(+), 238(-), 32(+), 430(-), 476(+), 593(-), 662(-)	3 copies at 15(+), 20(-), 913(+)	1 copy at 178(-)	1 copy at 183(-)	4 copies 169(±), 774(±)	1 copy at 913(+)	1 copy at 971(-)	NIL	NIL	NIL	2 copies at 265(-), 506(+)	NIL
CAU-G2-RT21	OsGLK21	NIL	4 copies at 467(+), 473(+), 846(-), 978(-)	10 copies at 232(+), 242(-), 287(+), 289(-), 302(+), 464(-), 728(+), 767(+), 785(+), 787(-)	3 copies at 1(-), 168(-), 866(-)	2 copies at 413(+), 959(-)	NIL	6 copies at 144(±), 402(±), 723(±)	1 copy at 866(+)	NIL	NIL	NIL	NIL	1 copy at 7(-)	NIL
CAU-G2-RT22	OsGLK22	3 copies at 364(-), 400(-), 985(+)	9 copies at 9(+), 103(+), 183(-), 370(+), 482(+), 494(+), 497(-), 724(-), 815(+)	10 copies at 39(-), 420(-), 445(+), 456(+), 509(-), 787(-), 812(-), 892(+), 917(+), 936(+)	4 copies at 256(-), 323(+), 418(-), 569(+)	2 copies at 575(-), 658(-)	NIL	4 copies at 150(±), 495(±)	2 copies at 256(-), 569(+)	NIL	NIL	2 copies at 519(±)	5 copies at 38(-), 419(-), 506(-), 786(-), 917(+)	1 copy at 82(-)	NIL
CAU-G2-RT23	OsGLK23	3 copies at 316(+), 373(+), 751(+)	13 copies at 92(-), 209(-), 269(+), 499(-), 522(+), 605, 638(-), 735(-), 767(-), 835(+), 874(+), 983(+), 985(-)	12 copies at 109(-), 198(-), 205(+), 304(+), 306(-), 349(+), 387(-), 507(+), 568(+), 774(-), 931(+), 942(-)	7 copies at 205(+), 618(-), 646(-), 721(-), 772(-), 920(-), 931(+)	2 copies at 76(-), 699(-)	1 copy at 710(-)	10 copies at 357(±), 472(±), 884(±), 907(±), 983(±)	2 copies at 618(-), 646(-)	NIL	NIL	NIL	3 copies at 205(+), 773(-), 931(+)	1 copy at 172(+)	NIL
CAU-G2-RT25	OsGLK25	6 copies at 10(-), 80(-), 361(-), 480(-), 609(-), 789(+)	11 copies at 5(+), 60(-), 380(+), 572(-), 577(+), 605(+), 664(+), 666(-), 679(+), 743(+), 907(+)	12 copies at 94(-), 101(+), 103(-), 139(-), 231(+), 236(+), 408(-), 488(+), 515(-), 723(-), 795(-), 914(-)	9 copies at 92(-), 211(+), 278(+), 327(+), 373(+), 406(-), 488(+), 698(+), 793(-)	3 copies at 186(-), 306(-), 335(-)	NIL	16 copies at 43(±), 146(±), 157(±), 508(±), 527(±), 563(±), 630(±), 664(±)	2 copies at 279(+), 698(+)	NIL	NIL	2 copies at 145(±)	5 copies at 93(-), 407(-), 488(+), 514(-), 794(-)	2 copies at 378(+), 413(+)	NIL
CAU-G2-RT26	OsGLK26	5 copies at 412(+), 451(-), 477(+), 633(+), 754(+)	8 copies at 43(-), 256(-), 388(-), 409(+), 515(+), 574(+), 623(-), 851(+)	5 copies at 166(+), 309(+), 341(-), 643(+), 678(+)	9 copies at 334(+), 428(-), 471(+), 531(-), 614(+), 678(+), 682(-), 733(-), 757(-)	1 copy at 592(-)	NIL	8 copies at 421(±), 669(±), 809(±), 856(±)	4 copies at 334(+), 471(+), 614(+), 757(-)	NIL	4 copies at 75(+), 91(+), 154(+), 914(-)	NIL	1 copy at 678(+)	1 copy at 180(-)	NIL
CAU-G2-RT27	OsGLK27	1 copy at 952(-)	9 copies at 74(+), 107(+), 168(+), 396(+), 424(+), 511(+), 552(+), 848(-), 950(+)	9 copies at 38(+), 40(-), 128(-), 215(+), 555(-), 623(+), 625(-), 816(+), 833(-)	6 copies at 1(-), 122(-), 192(-), 433(-), 864(-), 916(+)	1 copy at 349(-)	1 copy at 575(-)	14 copies at 43(±), 197(±), 376(±), 720(±), 800(±), 836(±), 962(±)	4 copies at 122(-), 192(-), 864(-), 916(+)	2 copies at 233(-), 711(+)	1 copy at 457(+)	NIL	NIL	1 copy at 26(-)	2 copies at 619(-), 712(+)
CAU-G2-RT28	OsGLK28	4 copies at 370(+), 655(+), 948(+), 973(-)	12 copies at 117(-), 122(-), 277(-), 345(+), 347(-), 398(-)	9 copies at 107(-), 129(+), 165(+), 250(-), 499(+), 603(+)	8 copies at 12(+), 156(-), 207(+), 561(+), 673(-), 830(+)	1 copy at 246(-)	NIL	18 copies at 5(±), 145(±), 187(±), 345(±), 358(±), 522(±), 643(±)	3 copies at 156(-), 208(+), 561(+)	NIL	NIL	NIL	4 copies at 129(+), 249(-), 499(+)	4 copies at 151(+), 697(+), 856(+)	NIL

			436(-), 467(-), 652(+), 663(+), 720(-), 904(-)	605(-), 675(-), 736(-)	853(-), 962(-)			820(±), 863(±)					674(-)	967(+)	
CAU-G2-RT29	OsGLK29	4 copies at 38(+), 44(+), 175(-), 179(-)	2 copies at 84(+), 227(-)	5 copies at 64(+), 483(+), 610(+), 617(-), 734(-)	6 copies at 56(+), 64(+), 87(-), 99(-), 188(-), 376(-)	1 copy at 390(-)	NIL	6 copies at 407(±), 582(±), 763(±)	4 copies at 56(+), 87(-), 99(-), 376(-)	NIL	1 copy at 540(-)	NIL	1 copy at 64(+)	1 copy at 369(+)	NIL
CAU-G2-RT30	OsGLK30	6 copies at 152(+), 231(-), 295(-), 376(+), 538(-), 792(+)	13 copies at 91(+), 102(+), 264(-), 333(-), 364(-), 420(+), 432(+), 570(+), 594(+), 640(+), 648(-), 675(+), 697(+)	12 copies at 24(+), 40(-), 88(-), 261(+), 269(+), 316(-), 447(-), 500(-), 591(-), 597(-), 672(-), 815(-)	6 copies at 4(-), 338(+), 603(-), 703(+), 808(-), 870(+)	1 copy at 417(+)	2 copies at 412(+), 579(+)	8 copies at 116(±), 194(±), 208(±), 836(±)	1 copy at 808(-)	2 copies at 303(-), 690(-)	NIL	2 copies at 313(±)	1 copy at 812(-)	2 copies at 600(+), 606(+)	NIL
CAU-G2-RT31	OsGLK31	1 copy at 732(-)	5 copies at 481(-), 813(-), 830(+), 950(-), 985(+)	12 copies at 134(-), 182(+), 191(+), 193(-), 271(-), 506(+), 555(-), 649(-), 822(-), 870(-), 920(-), 974(+)	9 copies at 252(-), 364(+), 416(-), 517(+), 607(-), 658(-), 675(-), 918(-), 974(+)	NIL	NIL	12 copies at 32(±), 144(±), 197(±), 208(±), 512(±), 989(±)	5 copies at 252(-), 416(-), 607(-), 658(-), 675(-)	NIL	NIL	4 copies at 304(±), 342(±)	5 copies at 554(-), 646(-), 821(-), 919(-), 974(+)	3 copies at 88(+), 127(+), 137(+)	NIL
CAU-G2-RT32	OsGLK32	NIL	12 copies at 309(+), 355(-), 431(-), 484(-), 712(-), 719(+), 732(-), 747(-), 829(-), 921(+), 981(+), 994(+)	7 copies at 176(-), 269(-), 275(-), 525(+), 533(-), 735(+), 939(+)	5 copies at 604(-), 735(+), 739(-), 894(+), 939(+)	2 copies at 476(-), 583(-)	NIL	8 copies at 52(±), 331(±), 579(±), 853(±)	1 copy at 604(-)	NIL	2 copies at 94(-), 117(+)	NIL	2 copies at 735(+), 939(+)	1 copy at 307(+)	2 copies at 296(-), 369(-)
CAU-G2-RT33	OsGLK33	1 copy at 512(-)	10 copies at 71(+), 231(+), 237(-), 272(+), 281(-), 521(-), 618(+), 700(-), 825(-), 892(+)	7 copies at 365(+), 367(-), 671(+), 676(-), 837(+), 949(-), 959(+)	5 copies at 509(+), 622(+), 671(+), 928(+), 959(+)	4 copies at 134(+), 153(+), 269(+), 905(-)	NIL	18 copies at 93(±), 99(±), 157(±), 198(±), 257(±), 289(±), 546(±), 578(±), 686(±)	2 copies at 509(+), 928(+)	NIL	NIL	NIL	3 copies at 671(+), 946(-), 959(+)	4 copies at 40(+), 46(+), 302(+), 862(+)	NIL
CAU-G2-RT34	OsGLK34	NIL	16 copies at 52(-), 60(-), 148(-), 159(+), 204(+), 251(-), 272(+), 416(+), 538(+), 541(-), 550(-), 561(-), 587(-), 604(+), 883(+), 889(-)	9 copies at 37(-), 42(-), 221(-), 351(-), 731(-), 739(-), 744(+), 824(-), 983(+)	6 copies at 143(+), 163(-), 281(-), 474(-), 530(+), 822(-)	2 copies at 535(+), 801(+)	NIL	10 copies at 64(±), 479(±), 503(±), 539(±), 624(±)	1 copy at 530(+)	NIL	NIL	2 copies at 736(±)	1 copy at 823(-)	4 copies at 157(+), 414(+), 967(-), 990(-)	NIL

CAU-G2-RT35	OsGLK35	8 copies at 102(-), 123(-), 188(-), 243(-), 364(+), 371(-), 415(+), 526(+)	8 copies at 42(+), 133(+), 177(-), 232(-), 303(-), 460(+), 798(-), 830(+)	6 copies at 97(-), 128(-), 381(-), 403(-), 449(-), 701(+)	15 copies at 52(+), 95(-), 110(+), 138(-), 169(-), 211(-), 224(-), 270(-), 424(+), 471(+), 546(+), 664(+), 717(+), 777(+), 913(+)	NIL	NIL	NIL	8 copies at 138(-), 169(-), 211(-), 224(-), 270(-), 546(+), 665(+), 913(+)	2 copies at 56(+), 475(+)	NIL	NIL	1 copy at 96(-)	NIL	NIL
CAU-G2-RT36	OsGLK36	NIL	9 copies at 164(+), 207(+), 269(+), 442(+), 550(+), 574(-), 597(+), 599(-), 698(+)	17 copies at 20(+), 83(-), 115(-), 202(+), 204(-), 338(+), 378(-), 404(-), 447(-), 494(+), 562(-), 612(+), 626(-), 679(-), 772(+), 932(-), 961(+)	11 copies at 81(-), 107(+), 113(-), 303(+), 445(-), 581(+), 612(+), 705(+), 772(+), 829(+), 961(+)	1 copy at 749(-)	NIL	16 copies at 244(±), 359(±), 415(±), 489(±), 597(±), 756(±), 983(±), 994(±)	NIL	1 copy at 653(-)	NIL	NIL	7 copies at 82(-), 114(-), 446(-), 561(-), 612(+), 772(+), 961(+)	1 copy at 659(+)	1 copy at 653(-)
CAU-G2-RT37	OsGLK37	2 copies at 244(-), 452(-)	6 copies at 178(+), 216(+), 223(+), 548(+), 615(+), 743(-)	4 copies at 230(-), 380(-), 487(+), 658(+)	5 copies at 135(+), 205(-), 263(-), 385(+), 444(-)	1 copy at 947(-)	NIL	6 copies at 42(±), 125(±), 591(±)	2 copies at 135(+), 444(-)	NIL	1 copy at 965(+)	NIL	1 copy at 377(-)	3 copies at 176(+), 311(+), 800(-)	1 copy at 200(+)
CAU-G2-RT38	OsGLK38	1 copy at 386(+)	3 copies at 23(+), 293(+), 523(+)	9 copies at 49(+), 93(-), 113(-), 117(-), 194(+), 212(+), 414(-), 714(-), 962(+)	2 copies at 54(+), 389(-)	NIL	NIL	2 copies at 128(±), 745(±)	2 copies at 54(+), 389(-)	1 copy at 887(-)	1 copy at 945(+)	NIL	NIL	2 copies at 35(-), 501(-)	NIL
CAU-G2-RT39	OsGLK39	6 copies at 60(+), 118(+), 310(-), 311(+), 386(+), 537(-)	10 copies at 91(+), 233(+), 287(+), 304(+), 356(+), 362(-), 475(-), 701(-), 740(+), 903(-)	4 copies at 284(-), 301(-), 413(-), 544(-)	6 copies at 23(+), 75(-), 173(+), 299(-), 542(-), 607(+)	NIL	NIL	8 copies at 45(±), 378(±), 438(±), 440(±)	1 copy at 75(-)	3 copies at 452(±), 494(+)	NIL	NIL	3 copies at 300(-), 410(-), 543(-)	1 copy at 175(-)	NIL
CAU-G2-RT40	OsGLK40	3 copies at 435(+), 450(-), 454(-)	12 copies at 91(-), 112(+), 290(+), 328(+), 330(-), 440(-), 481(-), 630(-), 643(-), 730(+), 864(-), 933(-)	8 copies at 355(-), 382(+), 472(+), 634(+), 850(-), 892(+), 894(-), 990(-)	10 copies at 48(+), 117(+), 124(+), 146(+), 315(-), 414(-), 472(+), 686(-), 848(-), 914(-)	3 copies at 431(-), 538(-), 940(+)	NIL	10 copies at 328(±), 370(±), 678(±), 774(±), 921(±)	4 copies at 125(+), 147(+), 315(-), 914(-)	NIL	NIL	2 copies at 252(±)	3 copies at 472(+), 849(-), 989(-)	1 copy at 438(-)	1 copy at 178(-)
CAU-G2-RT41	OsGLK41	5 copies at 15(-), 528(+),	6 copies at 98(+), 129(-), 202(-),	7 copies at 54(-), 109(+),	6 copies at 52(-), 71(+),	1 copy at 310(+)	NIL	4 copies at 459(±), 994(±)	NIL	1 copy at 891(+)	3 copies at 761(-), 838(-)	2 copies at 492(±)	3 copies at 53(-), 351(-),	1 copy at 300(+)	NIL

		584(+), 597(+), 643 (+)	434(+), 606(+), 680(+)	150(-), 165(-), 352(-), 358(+), 493(+)	178(+),317(+), 338(+), 358(+)							, 863(-)		358(+)		
CAU-G2-RT42	OsGLK42	4 copies at 504(+), 750(-), 910(+), 978 (-)	10 copies at 8 (+), 254(-), 488 (-), 519(-), 774(-), 800(+), 847(+), 854(+), 891(+), 898(+)	11 copies at 203(+), 378(+), 386(+), 515(-), 548(+), 557(-), 599(+), 744(-), 756(-), 788(+), 883(-)	4 copies at 203(+), 496(-), 513(-), 881(-)	NIL	1 copy at 829(+)	8 copies at 44(±), 412(±), 479(±), 723(±)	NIL	NIL	2 copies at 215(-), 243 (-)	NIL	4 copies at 203(+), 386(+), 514(-), 882 (-)	1 copy at 404(-)	2 copies at 82(-), 102(+)	
CAU-G2-RT43	OsGLK43	2 copies at 821(+), 943 (-)	8 copies at 79(+), 124(-), 323(+), 447(+), 449(-), 514(+), 779(-), 961(-)	4 copies at 85(-), 183(+), 320(-), 640(-)	6 copies at 6(-), 52(-), 83(-), 137(+), 369(+), 638(-)	1 copy at 94(+)	NIL	16 copies at 165(±), 447(±), 455(±), 508(±), 531(±), 557(±), 654(±), 669(±)	3 copies at 6(-), 52(-), 370(+)	NIL	NIL	NIL	4 copies at 84(-), 183(+), 319(-), 639 (-)	5 copies at 1(+), 216(+), 829(+), 911(-), 928 (-)	NIL	
CAU-G2-RT44	OsGLK44	4 copies at 57(-), 110(+), 137(-), 165 (-)	10 copies at 104(-), 145(-), 152(+), 199(+), 201(-), 516(+), 550(-), 706(+), 727(+), 781(-)	11 copies at 3(-), 43(-), 183(-), 485(-), 575(+), 771(+), 775(+), 845(-), 859(+), 861(-), 975(-)	7 copies at 64(-), 366(-), 429(+),454(+), 579(-), 625(-), 968(-)	NIL	NIL	12 copies at 85(±), 199(±), 326(±), 610(±), 738(±), 868(±)	1 copy at 64(-)	1 copy at 379(-)	NIL	2 copies at 29(±)	NIL	3 copies at 363(+), 704(+), 779(-)	NIL	
CAU-G2-RT45	OsGLK45	4 copies at 80(-), 159(-), 187(-), 590(-)	10 copies at 167(-), 174(+), 221(+), 223(-), 534(+), 568(-), 677(-), 715(+), 736(+), 789(-)	10 copies at 26(-), 66(-), 205(-), 503(-), 779(+), 783(+), 853(-), 875(+), 877(-), 922(-)	7 copies at 87(-), 388(-), 447(+),472(+), 597(-), 640(-), 985(-)	NIL	NIL	10 copies at 108(±), 221(±), 348(±), 625(±), 747(±)	1 copy at 87(-)	NIL	1 copy at 869(-)	2 copies at 52(±)	NIL	3 copies at 385(+), 713(+), 787(-)	NIL	
CAU-G2-RT46	OsGLK46	5 copies at 31(-), 61(+), 448(+), 459(-), 735(-)	6 copies at 161(+), 188(+), 191(-), 313(-), 319(+), 486(-)	6 copies at 273(-), 546(-), 572(+), 764(-), 807(+), 828(-)	10 copies at 69(-), 307(-), 333(-), 404(+), 521(-), 692(-), 745(+),807(+), 865(-), 952(-)	3 copies at 185(+), 655(+), 947(+)	NIL	2 copies at 189(±)	5 copies at 69(-), 307(-), 404(+), 692 (-), 745(+)	NIL	NIL	NIL	2 copies at 545(-), 807(+)	2 copies at 13(+), 326(-)	NIL	
CAU-G2-RT48	OsGLK48	2 copies at 43(-), 738(+)	6 copies at 277(-), 285(-), 653(-), 704(+), 727(+), 819(-)	6 copies at 16(+), 32(-), 659(+), 665(+), 815(-), 959(+)	NIL	3 copies at 301(+), 385(+), 696(+)	NIL	12 copies at 52(±), 69(±), 128(±), 292(±), 317(±), 869(±)	NIL	NIL	NIL	NIL	NIL	2 copies at 37(-), 828 (+)	NIL	
CAU-G2-RT49	OsGLK49	1 copy at 520(-)	6 copies at 187(+), 504(+), 517(+), 648(-), 752(-), 976(-)	3 copies at 151(+), 415(-), 838(+)	2 copies at 413(-), 995(+)	NIL	NIL	4 copies at 163(±), 220(±)	NIL	2 copies at 552(±)	3 copies at 765(+), 938(-), 941 (-)	NIL	1 copy at 414(-)	NIL	1 copy at 34(-)	
CAU-G2-RT50	OsGLK50	NIL	6 copies at 104(-), 316(-), 540(-), 637(+), 873(+), 879(+)	8 copies at 47(+), 126(+), 177(+), 387(+), 446(-), 486(-), 543(+), 645(-)	6 copies at 177(+), 210(-), 471(-), 595(+), 691(-), 862(-)	1 copy at 87(+)	NIL	8 copies at 325(±), 339(±), 463(±), 917(±)	3 copies at 210(-), 691 (-), 862(-)	1 copy at 67(-)	NIL	NIL	1 copy at 177(+)	3 copies at 51(-), 144(-), 877(+)	NIL	
CAU-G2-RT51	OsGLK51	4 copies at 35(-), 159(+), 724(-), 725 (+)	8 copies at 80(+), 180(-), 337(+), 448(+), 520(+), 634(-), 665(+), 938(-)	6 copies at 14(-), 150(+), 152(-), 272(+), 533(-), 817(+)	3 copies at 25(-), 458(+), 531(-)	5 copies at 125(+),182 (-), 229(-), 840(-), 892(-)	NIL	12 copies at 225(±), 248(±), 420(±), 435(±), 553(±), 568(±)	NIL	2 copies at 15(+), 926(-)	1 copy at 994(-)	2 copies at 552(±)	2 copies at 13(-), 532 (-)	4 copies at 210(-), 341 (-), 346(-), 663(+)	NIL	
CAU-G2-RT01	OSGLK52	NIL	4 copies at 143(-), 449(+), 461(+), 470(+)	5 copies at 138(+), 293(+), 502(+), 632(-), 640(-)	7 copies at 17(-), 226(-), 249(+), 573(-), 630(-), 738(-), 744(-)	1 copy at 970(-)	NIL	8 copies at 30(±), 156(±), 481(±), 816(±)	NIL	1 copy at 932(-)	NIL	NIL	1 copy at 631(-)	2 copies at 79(-), 221 (+)	1 copy at 932(-)	

Annexure II List of CG and SSR markers surveyed on LR 26 and LR 23

Primers Name						
PR1-1	PR81-6	RK-3	PR41-6	CR 42-1	HvSSR01-1	HvSSR05-21
PR1-2	PR81-7	RK-4	PR41-7	CR 42-2	HvSSR01-03	HvSSR05-22
PR1-3	PR83-1	RK-5	PR41-8	CR 42-3	HvSSR01-06	HvSSR05-23
PR2-1	PR83-2	LGD 1-1	PR42-1	CR 42-4	HvSSR01-07	HvSSR05-24
PR2-2	PR83-3	LGD 1-2	PR42-2	CR 42-5	HvSSR01-09	HvSSR05-25
PR2-3	PR83-4	LGD 1-3	PR42-3	CR 43-1	HvSSR01-10	HvSSR05-27
PR2-4	PR84-1	LGD 1-4	PR51-1	CR 43-2	HvSSR01-11	HvSSR05-33
PR2-5	PR84-2	LGD 1-5	PR51-2	CR 43-3	HvSSR01-12	HvSSR05-34
PR2-6	PR84-3	LGD 1-6	PR51-3	CR 43-4	HvSSR01-13	HvSSR05-37
PR2-7	PR84-4	LGD 1-7	PR52-1	CR 51-1	HvSSR01-14	HvSSR05-39
PR2-8	PR84-5	CAB2R-1	PR52-2	CR 51-2	HvSSR01-15	HvSSR05-40
PR3-1	PR91-1	CAB2R-2	PR53-1	CR 51-3	HvSSR01-17	HvSSR05-41
PR3-2	PR91-2	5'UTR NAC6	PR53-2	CR 61-1	HvSSR01-20	HvSSR05-43
PR3-3	PR91-3	NAC6-1	PR53-3	CR 61-2	HvSSR01-22	HvSSR05-44
PR3-4	PR91-4	NAC6-2	PR62-1	CR 61-3	HvSSR01-24	HvSSR05-45
PR3-5	PR91-5	AU1-1	PR62-2	ILA 1	HvSSR01-28	HvSSR05-48
PR3-6	PR91-6	AU1-2	PR62-3	ILA 2	HvSSR01-29	HvSSR05-49
PR3-7	PR92-1	AU1-3	PR62-4	ILA 3	HvSSR01-30	HvSSR05-50
PR4-1	PR93-1	AU1-4	PR62-5	ILA 4	HvSSR01-31	HvSSR05-51
PR4-2	PR93-2	AU9-1	PR62-6	ILA 5	HvSSR01-32	HvSSR05-53
PR4-3	PR93-3	AU9-2	PR63-1	ILA 6	HvSSR01-34	HvSSR05-54
PR5-1	PR101-1	AU9-3	PR63-2	ILA 7	HvSSR01-35	HvSSR05-57
PR5-2	PR101-2	AU9-4	PR63-3	PNH 1-1	HvSSR01-37	HvSSR05-59
PR5-3	PR101-3	PTF 1-1	PR63-4	PNH 1-2	HvSSR01-38	HvSSR06-09
PR5-4	PR101-4	PTF 1-2	PR71-1	PNH 1-3	HvSSR01-39	HvSSR06-10
PR6-1	PR101-5	PTF 1-3	PR71-2	PNH 1-4	HvSSR01-41	HvSSR06-12
PR6-2	PR102-1	PTF 1-4	PR81-1	PNH 1-5	HvSSR01-43	HvSSR06-13
PR6-3	PR102-2	PTF 1-5	PR81-2	PNH 1-6	HvSSR01-44	HvSSR06-16
PR7-1	PR102-3	PTF 1-6	PR81-3	PNH 1-7	HvSSR01-45	HvSSR06-17
PR7-2	PR102-4	PTF 1-7	PR81-4	RK-1	HvSSR01-46	HvSSR06-18
PR7-3	PR111-1	PTF C-1	PR81-5	RK-2	HvSSR01-47	HvSSR06-19
PR7-4	PR111-2	PTF C-2	HvSSR03-29	HvSSR04-12	HvSSR01-49	HvSSR06-20
PR7-5	PR111-3	PTF C-3	HvSSR03-30	HvSSR04-15	HvSSR01-51	HvSSR06-21
PR7-6	PR113-1	PTF C-4	HvSSR03-31	HvSSR04-17	HvSSR01-52	HvSSR06-22
PR7-7	PR113-2	PHR1-1	HvSSR03-32	HvSSR04-18	HvSSR01-53	HvSSR06-40
PR8-1	PR113-3	PHR1-2	HvSSR03-33	HvSSR04-19	HvSSR01-54	HvSSR06-41
PR8-2	PR113-4	PHR1-3	HvSSR03-34	HvSSR04-21	HvSSR01-56	HvSSR06-42
PR8-3	PR114-1	PHR1-4	HvSSR03-37	HvSSR04-27	HvSSR01-60	HvSSR06-43
PR8-4	PR114-2	PHR1-5	HvSSR03-38	HvSSR04-28	HvSSR01-62	HvSSR06-44
PR9-1	PR114-3	PHR1-6	HvSSR03-40	HvSSR04-31	HvSSR01-64	HvSSR06-45
PR9-2	PR114-4	PHR1-7	HvSSR03-41	HvSSR04-32	HvSSR01-65	HvSSR06-46
PR9-3	PR114-5	PHR2-1	HvSSR03-46	HvSSR04-33	HvSSR01-66	HvSSR06-47
PR9-4	PR114-6	PHR2-2	HvSSR03-47	HvSSR04-35	HvSSR01-67	HvSSR06-48
PR9-5	R63-1 (FoR)	PHR2-3	HvSSR03-51	HvSSR04-37	HvSSR01-70	HvSSR06-49
PR9-6	R63-2 (FoR)	PHR2-4	HvSSR03-53	HvSSR04-38	HvSSR01-71	HvSSR06-50
PR9-7	R63-3 (FoR)	PHR2-5	HvSSR03-54	HvSSR04-40	HvSSR01-73	HvSSR06-51
PR9-8	R63-4 (FoR)	PHR2-6	HvSSR03-55	HvSSR04-41	HvSSR01-76	HvSSR06-52
PR9-9	PR 0231	PHR2-7	HvSSR03-57	HvSSR04-46	HvSSR01-78	HvSSR06-53
PR10-1	PR 0232	K46-1	HvSSR03-59	HvSSR04-49	HvSSR01-79	HvSSR06-54
PR10-2	PR 0233	PR24-1	HvSSR03-60	HvSSR05-1	HvSSR01-80	HvSSR06-55
PR10-3	PR 0234	PR24-2	HvSSR03-62	HvSSR05-2	HvSSR01-81	HvSSR06-56
PR10-4	PR 0241	PR24-3	HvSSR03-64	HvSSR05-5	HvSSR01-82	HvSSR06-57
PR11-2	PR 0244	PR25-2	HvSSR03-67	HvSSR05-9	HvSSR01-85	HvSSR06-63
PR11-3	PR 0251	PR25-3	HvSSR03-68	HvSSR05-11	HvSSR01-86	HvSSR06-66
PR14-1	PR 0252	PR25-4	HvSSR03-69	HvSSR05-12	HvSSR01-87	HvSSR06-68
PR14-2	PR 0261	PR37-1	HvSSR03-70	HvSSR05-13	HvSSR01-88	HvSSR06-69
PR14-3	PR 0262	PR37-2	HvSSR03-71	HvSSR05-15	HvSSR01-92	HvSSR06-70
PR15-1	PR 0263	PR37-3	HvSSR03-72	HvSSR05-17	HvSSR01-94	HvSSR06-71

PR15-2	PR 0264	PR37-4	HvSSR03-73	HvSSR05-19	HvSSR02-2	HvSSR06-72
PR15-3	PR 0265	PR19-1	HvSSR03-74	HvSSR05-20	HvSSR02-3	HvSSR06-74
PR15-4	PR 0266	PR19-2	HvSSR03-77	HvSSR12-13	HvSSR02-5	HvSSR06-75
PR15-5	PR 0371	PR19-3	HvSSR03-80	HvSSR12-16	HvSSR02-10	HvSSR07-01
PR16-1	PR 0372	PR19-4	HvSSR03-82	HvSSR12-18	HvSSR02-11	HvSSR07-02
PR16-2	PR 0373	PR19-5	HvSSR03-85	HvSSR12-19	HvSSR02-12	HvSSR07-03
PR16-3	PR 0431	PR270-1	HvSSR03-86	HvSSR12-20	HvSSR02-14	HvSSR07-04
PR16-4	PR 0432	PR270-2	HvSSR03-87	HvSSR12-21	HvSSR02-15	HvSSR07-05
PR16-5	PR 0433	PR270-3	HvSSR03-88	HvSSR12-22	HvSSR02-16	HvSSR07-07
PR17-1	PR 0434	PR270-4	HvSSR03-93	HvSSR12-23	HvSSR02-17	HvSSR07-09
PR17-2	PR 0641	PR310-1	HvSSR10-27	HvSSR12-24	HvSSR02-24	HvSSR08-09
PR17-3	PR 0642	PR310-2	HvSSR10-29	HvSSR12-26	HvSSR02-27	HvSSR08-10
PR17-4	PR 0643	PR310-3	HvSSR10-30	HvSSR12-28	HvSSR02-29	HvSSR08-11
PR21-1	PR 0644	PR310-4	HvSSR10-33	HvSSR12-30	HvSSR02-34	HvSSR08-14
PR21-2	PR 0645	PR110-1	HvSSR10-34	HvSSR12-34	HvSSR02-48	HvSSR08-15
PR21-3	PR 0651	PR110-2	HvSSR10-35	HvSSR12-35	HvSSR02-50	HvSSR08-16
PR21-4	PR 0652	PR110-3	HvSSR10-37	HvSSR12-36	HvSSR02-71	HvSSR08-17
PR21-5	PR 0653	PR110-4	HvSSR10-38	HvSSR12-37	HvSSR02-72	HvSSR08-18
PR21-6	PR 0654	PR670-1	HvSSR10-42	HvSSR12-38	HvSSR02-73	HvSSR08-19
PR21-7	CR 1-1	PR670-2	HvSSR10-45	HvSSR12-39	HvSSR02-74	HvSSR08-23
PR22-1	CR 1-2	PR670-3	HvSSR11-01	HvSSR12-43	HvSSR02-76	HvSSR08-24
PR22-2	CR 1-3	PR670-4	HvSSR11-03	HvSSR09-26	HvSSR02-77	HvSSR08-28
PR22-3	CR 1-4	PR680-1	HvSSR11-04	HvSSR09-27	HvSSR02-78	HvSSR08-29
PR22-4	CR 2-1	PR680-2	HvSSR11-07	HvSSR09-30	HvSSR02-79	HvSSR08-31
PR31-1	CR 2-2	PR680-3	HvSSR11-10	HvSSR09-36	HvSSR02-80	HvSSR08-33
PR31-2	CR 2-3	PR680-4	HvSSR11-11	HvSSR09-39	HvSSR02-81	HvSSR08-34
PR31-3	CR 2-4	PR680-5	HvSSR11-12	HvSSR09-40	HvSSR02-82	HvSSR08-35
PR33-1	CR 2-5	PR96-1	HvSSR11-13	HvSSR09-42	HvSSR02-83	HvSSR08-40
PR33-2	CR 3-1	PR96-2	HvSSR11-15	HvSSR09-43	HvSSR02-85	HvSSR08-41
PR33-3	CR 3-2	PR96-3	HvSSR11-18	HvSSR09-44	HvSSR02-86	HvSSR08-44
PR33-4	CR 3-3	PR96-4	HvSSR11-19	HvSSR09-45	HvSSR03-02	HvSSR08-45
PR34-1	CR 4-1	AR1-1	HvSSR11-20	HvSSR09-48	HvSSR03-03	HvSSR08-46
PR34-2	CR 4-2	AR1-2	HvSSR11-21	HvSSR09-50	HvSSR03-04	HvSSR08-47
PR34-3	CR 4-3	AR1-3	HvSSR11-22	HvSSR09-53	HvSSR03-06	HvSSR08-48
PR34-4	CR 4-4	AR1-4	HvSSR11-23	HvSSR09-55	HvSSR03-07	HvSSR08-49
PR34-5	CR 11-1	AR1-5	HvSSR11-25	HvSSR09-56	HvSSR03-08	HvSSR08-50
PR35-1	CR 11-2	AR1-6	HvSSR11-27	HvSSR10-02	HvSSR03-10	HvSSR08-51
PR35-2	CR 12-1	FR033-1	HvSSR11-28	HvSSR10-03	HvSSR03-11	HvSSR09-01
PR35-3	CR 12-2	FR033-2	HvSSR11-30	HvSSR10-05	HvSSR03-12	HvSSR09-02
PR35-4	CR 21-1	FR033-3	HvSSR11-48	HvSSR10-06	HvSSR03-13	HvSSR09-03
PR35-5	CR 21-2	FR033-4	HvSSR11-50	HvSSR10-07	HvSSR03-14	HvSSR09-06
PR36-1	CR 21-3	FR71-1	HvSSR11-58	HvSSR10-08	HvSSR03-15	HvSSR09-07
PR36-2	CR 22-1	AR51-1	HvSSR11-75	HvSSR10-09	HvSSR03-17	HvSSR09-09
PR36-3	CR 22-2	AR51-2	HvSSR11-76	HvSSR10-18	HvSSR03-18	HvSSR09-11
PR36-4	CR 22-3	AR51-3	HvSSR12-01	HvSSR10-21	HvSSR03-19	HvSSR09-18
PR41-1	CR 22-4	AR51-4	HvSSR12-04	HvSSR10-25	HvSSR03-21	HvSSR09-19
PR41-2	CR 41-1	AR51-5	HvSSR12-08		HvSSR03-23	HvSSR09-22
PR41-3	CR 41-2	HvSSR03-25	HvSSR12-09		HvSSR04-04	HvSSR09-23

Annexure III

Frequency of amino acid in OsGLK (given in percent).

Gene name	Ala	Cys	Asp	Glu	Phe	Gly	His	Ile	Lys	Leu	Met	Asn	Pro	Gln	Arg	Ser	Thr	Val	Trp	Tyr
OsGLK1	11.2	1.31	7.66	5.47	2.84	7.44	3.06	3.5	3.3	5.5	3.06	1.8	13	4.4	4.6	7.7	3.1	8.32	1.8	0.88
OsGLK2	16.1	1.38	6.1	4.92	1.97	6.3	3.94	2.17	4.9	6.3	1.97	1	16	3.2	3.9	6.7	3.4	6.3	1.8	1.38
OsGLK3	13	2.6	6.69	5.95	2.23	8.18	2.6	1.12	3	6.7	2.23	1.9	14	1.1	6	10	4.1	7.06	0.7	1.12
OsGLK4	4.53	2.36	9.25	4.92	2.36	5.51	2.36	4.33	7.3	9.8	3.94	4.3	5.5	4.9	4.9	9.8	3.9	7.09	0.8	1.97
OsGLK5	9.04	1.29	3.1	5.43	2.84	7.24	4.65	3.1	3.4	7.2	2.33	4.9	7.8	4.4	3.9	15	5.4	4.91	0.5	3.36
OsGLK6	6.61	2.35	5.97	8.1	2.35	3.2	2.56	3.62	7.9	9	2.99	3.8	5.3	6.8	3.6	15	5.3	3.2	0.2	2.35
OsGLK7	7.6	1.72	4.9	5.15	1.72	8.33	5.15	4.17	4.2	7.6	4.41	3.4	5.4	6.1	9.3	8.1	5.2	5.39	1	1.23
OsGLK8	9.38	1.73	7.16	4.94	1.98	11.4	3.7	2.22	5.4	8.4	5.68	1.7	5.2	6.2	6.2	6.4	3.7	5.68	0.5	2.47
OsGLK10	10.8	0.96	5.78	7.71	1.69	6.02	3.61	1.45	6	6.3	1.45	1.7	9.6	7.7	6	11	4.8	5.3	1	0.96
OsGLK14	5.19	1.98	3.7	6.42	2.22	7.16	4.94	2.96	6.4	13	3.7	4.9	4.4	7.9	4.2	11	4.7	4.2	0.5	1.23
OsGLK15	7.87	0.93	6.25	5.09	1.62	4.4	3.94	3.7	6.3	7.2	3.24	5.6	7.2	6	3.5	13	6.9	4.17	0.9	1.85
OsGLK16	11.8	1.18	5.12	5.12	2.76	7.09	2.36	5.12	4.9	8.5	4.33	4.5	4.7	2.8	6.7	7.1	5.5	6.89	0.6	2.95
OsGLK17	14.2	1.49	5.72	6.22	1.24	6.97	1.74	2.24	4.5	8.7	2.49	1.5	6.7	5	8	11	4.2	6.47	1	0.75
OsGLK18	13.9	1.24	3.23	4.48	1.49	10.2	3.98	2.49	2.5	9.5	3.23	3	8.7	3.7	4.5	10	7	4.98	0.5	1
OsGLK19	7.37	3.44	3.44	6.14	1.97	16	2.21	3.93	4.2	7.6	3.69	1.7	5.9	4.9	5.4	11	3.9	4.67	1	1.23
OsGLK21	8.4	0	3.7	4.44	3.7	23.5	5.93	1.48	2.2	7.7	3.46	1.7	8.6	3	5.2	5.4	4.4	4.94	1	1.23
OsGLK22	5.76	1.2	4.8	6.95	2.4	7.43	3.84	3.84	6	11	1.68	5	2.6	8.9	5.3	9.8	6	4.08	0.5	2.88
OsGLK23	8.19	0.99	10.2	7.44	3.72	8.93	1.99	3.72	5.7	7.2	3.97	3.2	6.7	6	3	7.9	2.5	6.7	0.5	1.49
OsGLK25	10.4	0.2	7.68	6.89	3.94	10.8	4.92	1.77	4.1	6.5	3.35	4.5	3.9	3.2	7.3	10	4.1	4.72	0.8	0.59
OsGLK26	7.65	0.99	2.96	3.46	1.73	3.21	5.43	4.94	4.9	9.6	3.7	6.4	6.7	10	4.2	8.4	5.9	5.43	1	2.96
OsGLK27	9.65	1.18	4.13	6.3	1.97	7.48	2.36	3.15	3.9	7.1	2.56	6.1	5.7	5.9	3.9	12	7.3	6.3	1.4	1.38
OsGLK28	5.34	0.59	7.72	6.23	2.37	7.72	2.67	5.34	5.3	9.2	1.19	4.8	7.1	5.3	6.2	12	3.3	5.04	0.3	1.78
OsGLK29	6.88	0.53	5.82	4.23	2.38	9.52	3.97	2.91	5.8	7.7	6.35	1.9	5.8	6.9	4.8	12	4.5	5.29	0.8	1.85
OsGLK30	5.72	1.99	4.98	6.47	2.99	4.23	2.49	4.98	4.7	7.7	2.24	4.7	5.5	4.7	5.7	16	6.7	5.22	1.2	1.49
OsGLK31	5.81	1.45	4.77	8.71	2.49	4.77	3.32	3.32	7.7	8.9	1.45	3.3	7.3	6.4	3.3	16	3.5	5.6	0.2	1.24
OsGLK32	7.23	0.93	5.59	6.76	1.63	6.53	1.86	3.96	4.4	7.7	1.63	5.6	8.2	6.8	4.4	13	6.1	4.66	1.2	2.33
OsGLK33	9.16	1.49	3.47	6.68	1.73	8.17	5.94	3.22	3.5	11	2.48	3.7	5	8.4	6.2	11	4.5	2.72	0.5	1.24
OsGLK34	14.8	2.11	3.63	4.53	2.72	10.3	5.44	2.11	2.4	9.1	3.63	3.6	8.5	3.6	5.7	8.2	4.2	3.32	0.9	1.21
OsGLK35	7.55	1.13	6.04	4.91	4.15	7.55	4.53	4.15	6.8	11	3.02	2.3	4.5	5.7	4.5	9.8	4.9	4.91	0.8	1.89
OsGLK36	8.6	0.74	4.67	6.14	1.23	10.6	2.7	3.44	4.2	8.1	1.97	3.9	9.1	6.9	4.9	11	5.9	4.18	0.7	1.47
OsGLK37	13	0.85	5.77	2.56	1.28	13.5	3.85	1.5	1.3	8.3	2.78	1.5	7.7	1.9	6.4	15	6	4.27	1.3	1.71
OsGLK38	12.4	0.95	5.71	8.57	1.59	7.94	3.49	1.9	3.5	8.3	2.22	2.2	2.9	7.6	8.3	13	3.5	4.44	0.3	1.59
OsGLK39	6.9	1.15	4.6	7.66	3.07	8.81	3.45	3.83	7.3	11	3.07	1.5	5.8	6.5	5	9.6	3.8	5.36	0.4	1.53
OsGLK40	7.18	0.99	4.46	6.44	2.23	12.1	2.48	2.97	4.5	7.9	2.23	4.5	5.9	5.7	7.4	11	5	4.21	1	1.49
OsGLK41	12.2	0.98	3.74	2.36	2.36	15.2	5.31	1.57	2	6.9	3.54	1.8	8.7	1.8	6.3	14	4.3	4.53	1.6	1.18
OsGLK42	6.33	1.81	3.62	5.88	4.98	5.88	6.79	4.98	5	6.8	4.98	7.7	3.2	3.2	7.2	3.6	9.1	4.98	3.2	0.9
OsGLK43	7.84	2.21	4.17	6.62	0.74	10.1	4.9	2.21	2.9	8.3	3.19	2.7	5.2	4.7	8.8	12	6.4	3.92	1	2.45
OsGLK44	7.16	1.23	3.95	8.15	1.73	9.63	4.2	2.72	4	7.4	1.98	4.2	4.9	3.5	8.2	15	4.7	3.95	1.7	2.22
OsGLK45	11.1	0.99	3.96	7.18	1.49	9.65	5.2	3.47	4	8.4	2.48	3.2	4.2	4	7.2	12	4.2	3.96	1.5	1.98
OsGLK46	17.5	1.23	4.68	6.4	1.72	7.39	2.46	1.72	4.9	6.7	2.46	1.7	5.2	5.7	6.7	11	4.4	5.67	1.5	0.74

OsGLK48	9.45	0.2	6.5	6.69	2.76	9.65	2.95	2.36	4.1	7.3	3.35	3.9	5.7	3.9	7.9	11	5.7	4.53	1	0.98
OsGLK49	7.88	1.71	3.77	5.48	5.82	6.85	4.79	4.11	5.1	6.9	6.16	2.7	4.1	5.5	9.6	8.2	3.1	4.11	1	3.08
OsGLK50	10.3	0.74	3.69	2.22	2.46	10.3	7.39	3.45	3.9	8.4	2.71	3.5	7.1	6.4	6.2	7.6	6.4	4.68	0.7	1.72
OsGLK51	8.53	3.72	5.69	5.69	1.97	10.7	3.28	2.84	2.2	7.9	2.19	3.5	6.4	4.6	7.2	11	5.3	4.81	0.7	1.75
OsGLK52	18	0.39	6.11	6.11	1.18	8.09	5.13	2.76	3.9	7.1	1.97	2.8	8.5	4.9	4.1	7.1	4.5	4.34	0.8	2.17
Avg.	9.56	1.33	5.27	5.85	2.28	8.73	3.81	3.1	4.5	8.1	3	3.4	6.8	5.2	5.8	11	4.9	5.03	0.9	1.67

Annexure IV

Field data of F₆ plants derived from LR23 x LR 26 cross

Plant no.	TN 30	TN 60	50%DTF	PH	PL	LA	PN	FG	100 SW	F %	GYPP	P content (µg/mg of plant sample)	PDW (Harvest)	PUE at harvest	PDW 60 DAT	PUE at 60
143(2)	3.08	8.57	78.13	96.59	23.62	42.73	7.79	568.92	0.26	77.89	12.05	1.45	31.81	22.00	21	14.53
121(2)	2.68	6.11	99.00	132.52	25.31	49.19	5.99	492.12	1.12	62.28	8.20	1.23	40.05	32.45	10	8.10
239(5)	4.28	11.31	92.40	108.72	18.91	41.37	3.99	115.52	0.71	30.33	1.58	1.66	19.77	11.93	17	10.26
174(5)	2.81	7.97	77.13	107.45	24.82	51.56	7.05	362.52	2.85	60.40	6.71	1.23	22.70	18.45	15	12.20
25(3)	2.88	6.51	76.20	98.12	18.71	38.57	6.19	248.92	1.33	51.92	4.78	1.55	22.75	14.66	22	14.18
31(1)	2.61	7.17	78.53	111.45	23.15	51.46	6.05	378.32	3.37	63.84	9.01	1.67	24.86	14.88	9	5.39
13(1)	2.41	6.17	76.33	102.05	21.75	48.16	5.45	348.32	3.17	70.65	7.67	1.60	20.74	12.93	10	6.23
30(4)	1.81	7.17	87.33	116.65	23.35	46.42	6.45	393.12	3.40	55.47	9.29	1.22	25.72	21.07	3	2.46
150(1)	2.68	6.57	95.53	117.58	17.22	40.45	4.99	520.12	1.00	97.51	15.21	1.01	27.27	27.02	5	4.95
76(3)	2.01	5.31	85.13	110.72	22.57	42.89	4.85	408.42	2.50	45.32	5.77	1.21	19.29	15.97	22	18.22
197(5)	2.81	9.57	85.53	104.25	21.15	36.02	8.25	448.32	3.22	68.40	9.75	1.34	22.56	16.84	8	5.97
137(5)	4.08	11.31	86.80	129.92	20.31	49.57	7.39	536.12	1.20	66.89	9.36	1.67	37.57	22.49	23	13.77
197(3)	2.81	9.57	87.33	107.85	22.95	36.26	8.65	572.52	3.15	70.05	11.69	1.19	29.56	24.75	5	4.19
124(3)	2.48	5.71	84.40	129.92	24.97	46.11	5.19	638.92	2.13	80.55	17.11	1.53	40.41	26.49	26	17.05
47(2)	2.41	7.37	79.33	113.85	23.82	46.18	5.65	281.72	2.89	52.50	5.91	1.01	20.74	20.55	4	3.96
69(4)	2.08	6.57	82.53	106.79	22.75	37.89	4.59	365.32	0.22	78.72	7.98	1.62	22.35	13.82	31	19.16
107(5)	2.41	8.37	87.53	105.25	26.15	45.80	7.85	361.92	3.12	56.95	8.27	0.98	30.58	31.20	21	21.43
112(3)	2.41	6.57	86.53	115.45	24.15	51.82	7.25	434.32	3.52	67.00	10.75	0.90	35.42	39.20	8	8.86
27(5)	2.68	7.91	86.40	110.92	22.24	39.21	4.59	309.52	1.37	59.25	6.01	1.02	24.99	24.44	22	21.52
203(1)	2.28	5.51	93.60	115.52	20.77	50.87	3.79	338.32	1.28	64.14	6.25	1.35	24.39	18.02	11	8.13
69(5)	2.21	7.71	81.53	92.32	22.77	32.90	6.65	370.22	2.44	53.80	4.89	1.25	24.73	19.83	12	9.62

123(5)	2.08	5.51	83.40	103.72	18.31	28.93	4.39	183.92	1.78	75.96	4.42	1.45	14.31	9.90	20	13.83
4(1)	2.41	6.77	85.33	106.85	21.15	53.40	4.85	340.52	3.20	79.70	7.59	0.81	18.76	23.14	9	11.10
244(1)	3.01	7.97	95.13	127.45	23.48	58.06	8.05	481.92	2.89	48.20	8.85	0.74	34.80	46.73	4	5.37
185(4)	2.08	4.91	81.60	105.32	20.31	40.57	4.59	290.92	1.54	69.32	6.17	1.54	20.21	13.14	23	14.95
128(2)	2.21	8.37	92.13	117.65	23.42	44.56	8.05	429.92	3.52	52.35	10.65	1.30	30.46	23.43	4	3.08
32(4)	2.61	7.77	78.33	110.05	23.75	46.86	7.05	383.32	3.46	64.75	9.39	1.08	25.10	23.34	7	6.51
172(1)	2.01	6.77	84.13	119.25	22.42	40.14	6.65	385.92	3.41	74.61	9.33	1.23	25.46	20.63	4	3.24
120(4)	2.48	5.51	84.40	99.12	22.17	38.23	5.59	406.12	1.70	61.90	9.18	1.17	26.11	22.35	5	4.28
245(5)	2.88	8.71	98.40	124.32	22.31	45.30	6.79	305.32	0.99	44.75	4.76	1.60	29.69	18.50	9	5.61
165(3)	1.81	6.17	85.53	117.65	24.68	215.80	6.05	478.12	3.00	57.21	9.13	1.26	29.84	23.67	7	5.55
5(4)	1.68	5.24	74.53	100.59	19.88	43.52	3.65	470.82	0.16	80.25	9.68	0.88	16.80	19.16	33	37.63
63(1)	1.88	7.97	85.13	101.79	24.88	50.75	6.99	825.72	0.31	71.55	17.47	1.70	39.15	23.07	35	20.62
142(4)	3.68	8.57	74.53	107.08	19.88	35.05	5.49	267.12	0.28	72.64	6.30	1.74	22.07	12.71	22	12.67
142(1)	3.08	6.71	74.40	116.32	19.51	37.17	5.19	139.92	1.30	53.89	2.72	1.41	20.51	14.59	28	19.91
175(5)	2.68	9.97	100.33	130.98	25.08	57.29	8.19	1061.72	0.48	83.17	23.95	1.34	62.57	46.70	69	51.49
49(5)	1.88	5.37	79.93	111.58	22.15	40.41	4.99	366.72	0.50	77.61	9.10	0.94	23.01	24.40	23	24.39
149(5)	2.61	6.84	83.20	118.52	25.08	36.13	5.72	302.22	2.93	55.62	8.33	1.14	22.45	19.67	16	14.02
185(5)	2.11	6.09	76.75	97.82	20.72	34.42	5.27	196.32	2.59	68.43	4.53	0.74	12.88	17.29	7	9.40
157(1)	2.88	6.57	74.53	107.38	19.75	46.69	3.19	505.92	0.20	84.81	10.52	0.88	23.51	26.81	17	19.38
109(4)	2.61	7.91	85.53	126.12	21.84	32.86	6.45	535.22	2.74	80.49	9.17	1.25	19.87	15.93	25	20.04
157(2)	2.81	8.71	79.33	103.52	18.91	26.96	6.25	391.22	2.68	64.58	5.92	1.53	22.51	14.76	17	11.15
189(2)	1.81	5.91	87.33	109.32	25.71	41.74	5.05	549.22	2.46	63.91	8.27	1.51	27.97	18.50	9	5.95
218(2)	2.88	7.01	74.40	90.52	21.44	35.05	2.89	213.02	1.76	64.53	5.04	1.54	17.60	11.44	18	11.70
22(4)	3.21	11.44	93.80	111.52	24.08	88.89	7.72	829.02	2.31	67.97	16.73	1.43	51.77	36.14	8	5.58
1(4)	2.81	5.84	75.00	100.12	19.48	38.79	4.92	288.42	2.08	67.18	4.70	1.05	14.33	13.66	16	15.25
21(5)	2.88	5.11	81.80	115.72	22.31	35.95	4.59	211.32	0.98	38.95	3.33	1.31	19.07	14.52	16	12.18
89(1)	2.21	4.71	78.53	91.52	24.64	40.88	4.25	341.42	2.56	48.97	4.63	1.37	20.25	14.82	9	6.59

146(1)	2.61	9.51	90.53	109.72	20.51	42.18	7.65	517.62	2.33	60.39	7.17	1.26	47.87	37.97	6	4.76
143(1)	2.01	8.11	86.73	93.32	20.44	38.26	6.25	557.42	2.61	84.36	9.08	1.18	30.17	25.54	23	19.47
103(5)	2.81	6.64	77.60	131.32	23.95	48.61	5.12	439.42	2.45	65.61	9.47	0.88	27.83	31.73	52	59.29
253(1)	2.88	6.51	88.60	110.92	22.24	41.43	6.39	460.32	1.64	70.35	10.10	0.98	28.65	29.32	21	21.49
242(4)	3.01	9.04	95.60	121.72	24.55	56.21	6.12	481.02	2.00	55.39	7.80	1.28	50.45	39.51	35	27.41
163(4)	2.41	5.91	76.53	85.52	23.51	42.28	5.45	507.02	2.75	67.82	8.60	1.38	26.79	19.46	21	15.25
56(2)	2.01	7.51	85.73	101.12	24.57	39.10	7.45	794.42	2.84	78.68	15.42	1.17	34.95	29.76	11	9.36
135(1)	1.88	6.51	98.80	90.72	21.64	42.01	7.59	641.92	1.54	85.54	13.35	1.21	29.15	24.13	25	20.70
186(2)	1.88	7.17	95.73	111.58	22.68	41.21	5.99	655.12	0.31	76.97	14.09	0.74	35.53	47.70	13	17.46
62(4)	1.81	6.51	81.66	96.45	22.88	40.22	6.65	196.52	3.08	36.12	4.68	0.79	20.64	26.13	9	11.39
35(3)	2.88	7.97	80.13	118.78	22.95	48.81	6.99	739.92	0.71	84.66	18.83	1.30	39.99	30.75	23	17.69
14(3)	2.08	3.31	74.40	101.72	18.44	39.99	3.79	445.12	1.32	78.66	8.34	1.17	21.65	18.54	26	22.26
227(1)	2.08	4.97	79.93	98.99	23.15	41.97	4.39	358.52	0.62	60.55	9.41	1.31	20.71	15.77	19	14.47
91(3)	2.21	7.71	83.73	95.72	22.51	45.68	8.05	642.79	2.26	64.87	8.97	1.99	38.87	19.55	15	7.54
26(3)	3.35	7.24	74.53	115.58	20.33	32.42	3.32	282.82	0.42	78.43	7.03	1.22	21.70	17.77	17	13.92
46(2)	3.48	8.31	97.60	111.52	22.97	40.95	8.19	499.52	1.68	78.01	11.15	1.35	36.45	26.93	15	11.09
75(4)	1.61	5.77	87.13	127.45	23.02	53.48	5.85	375.52	3.28	61.61	8.55	0.94	28.68	30.41	13	13.78
237(1)	2.01	7.57	92.93	120.25	22.68	54.00	7.25	383.12	2.86	48.01	7.41	0.86	26.18	30.31	41	47.47
31(5)	2.21	5.77	78.13	109.85	22.95	48.10	4.05	312.72	3.35	73.46	7.53	1.46	16.74	11.47	8	5.48
247(4)	1.81	7.51	101.33	115.12	22.57	45.00	5.85	567.39	2.18	63.03	7.33	1.27	31.23	24.52	9	7.07
238(3)	1.88	5.31	98.20	120.92	21.11	46.39	5.39	244.72	0.97	46.69	3.80	1.55	21.39	13.79	7	4.51
197(3)	2.81	9.57	87.33	107.85	22.95	36.26	8.65	572.52	3.15	70.05	11.69	1.19	29.56	24.75	5	4.19
93(3)	3.88	5.84	75.40	103.85	21.44	41.01	4.39	543.22	1.44	68.03	10.83	1.43	38.61	26.95	13	9.07
119(2)	3.08	6.77	80.93	121.78	22.48	40.35	5.39	482.32	0.14	59.95	9.74	1.20	30.65	25.54	17	14.17
151(1)	2.61	7.17	91.93	146.25	29.88	62.48	6.85	437.92	3.44	51.41	10.49	1.60	48.94	30.50	38	23.68
171(3)	2.81	9.37	78.73	97.65	23.42	34.66	7.85	416.52	3.31	68.00	9.55	0.98	23.52	24.00	11	11.22

98(5)	3.08	6.91	88.20	125.72	24.71	44.71	5.39	577.72	1.51	78.11	11.85	1.09	36.91	33.86	22	20.18
120(5)	1.41	6.31	81.33	106.12	22.97	42.04	5.45	597.19	2.74	83.82	10.48	1.17	28.93	24.77	5	4.28
83(3)	2.88	9.11	88.40	125.72	23.84	45.11	7.79	623.52	1.41	70.64	12.20	1.11	39.47	35.56	22	19.82
151(3)	3.61	7.24	90.00	122.12	25.15	44.29	6.52	471.62	2.44	74.57	10.13	1.20	33.25	27.71	16	13.33
165(3)	1.81	6.17	85.53	117.65	24.68	215.80	6.05	478.12	3.00	57.21	9.13	0.99	29.84	30.14	16	16.16
182(2)	2.01	6.31	87.33	116.72	25.17	42.26	5.85	480.42	2.21	55.33	6.07	0.85	33.75	39.68	14	16.46
52(1)	2.61	10.64	74.60	84.72	19.88	25.91	7.12	474.82	2.45	78.29	10.22	1.51	18.05	11.94	12	7.94
226(4)	2.68	7.57	82.53	98.79	23.08	36.93	5.99	460.72	0.39	77.40	10.60	1.40	24.05	17.18	13	9.29
58(2)	2.28	5.51	93.40	128.12	24.84	41.01	5.19	605.52	1.68	78.66	13.46	1.21	33.39	27.65	4	3.31
42(5)	1.81	5.57	80.73	111.05	25.75	47.12	5.65	348.12	3.22	60.46	7.87	1.31	23.60	17.97	5	3.81
86(5)	3.61	8.91	97.13	132.12	24.04	40.84	7.85	926.22	2.79	73.32	17.91	1.21	52.89	43.79	12	9.94
72(1)	2.68	9.51	83.40	110.32	24.11	46.31	8.99	866.92	1.82	84.81	20.42	1.14	46.75	40.95	28	24.53
95(3)	2.81	8.37	82.13	124.65	24.28	41.09	8.05	272.52	3.32	35.34	6.67	1.47	30.64	20.81	41	27.85
173(2)	1.81	7.67	89.83	121.95	26.38	41.12	6.65	415.52	3.11	75.03	8.65	1.33	27.19	20.44	13	9.77
231(2)	1.76	5.91	84.73	116.62	22.24	36.32	4.70	440.02	2.65	76.30	6.86	1.27	19.33	15.22	9	7.09
108(3)	2.01	7.17	84.73	121.85	25.02	46.08	6.65	297.92	3.28	50.32	7.49	1.27	28.06	22.03	19	14.92
71(3)	3.01	7.84	80.20	115.12	23.42	33.87	6.12	364.62	2.30	68.88	7.11	1.30	21.01	16.16	10	7.69
46(3)	2.68	6.11	80.80	100.72	22.91	33.71	5.19	233.12	1.30	50.89	4.40	1.45	19.97	13.81	7	4.84
216(2)	2.61	5.64	76.60	82.92	22.55	34.73	5.12	334.62	2.48	69.08	7.29	1.59	12.97	8.15	12	7.54
232(1)	2.88	6.17	81.73	88.19	21.68	36.33	4.79	417.12	0.46	72.98	10.00	1.56	19.87	12.70	30	19.17
159(3)	3.08	8.77	83.53	109.58	20.35	36.77	5.99	575.52	0.63	84.68	14.43	1.46	30.81	21.11	25	17.14
160(2)	1.81	5.91	88.53	107.32	24.44	53.54	6.45	676.19	2.22	52.91	9.27	1.27	44.09	34.61	9	7.07
99(3)	1.88	7.51	99.40	107.52	15.77	38.95	5.39	313.52	1.10	81.10	5.23	1.00	20.25	20.33	5	5.02
108(4)	2.28	6.97	87.93	114.78	24.55	44.35	5.59	466.32	0.56	76.94	11.58	1.46	32.87	22.53	18	12.34
223(4)	3.01	7.64	85.00	86.12	20.82	33.13	6.52	238.22	2.17	62.87	4.10	0.89	11.95	13.42	20	22.47
224(2)	2.81	8.24	91.60	103.92	22.22	44.15	7.92	550.02	1.64	73.30	6.65	1.82	32.45	17.83	29	15.93
66(5)	2.61	5.84	74.00	83.12	21.55	33.13	5.12	337.62	2.61	76.64	7.92	1.14	14.11	12.36	6	5.26

105(1)	2.68	8.07	83.53	104.34	21.22	688.00	5.49	456.12	0.45	86.04	10.78	0.88	23.22	26.48	24	27.37
198(4)	1.41	5.91	98.33	133.92	22.99	40.54	6.25	691.99	2.63	74.76	11.91	1.27	38.15	29.95	12	9.42
63(2)	2.61	6.24	91.60	98.12	22.28	56.57	5.12	366.22	2.05	69.42	6.01	1.25	23.49	18.79	12	9.60
124(2)	2.26	6.66	90.73	131.12	27.99	42.80	5.95	948.52	2.89	86.01	19.27	1.21	48.06	39.79	7	5.80
232(5)	2.21	7.11	80.53	92.52	22.44	41.66	5.45	383.22	2.52	49.53	5.33	1.35	21.61	15.97	8	5.91
115(2)	2.68	4.91	82.40	118.92	21.64	38.29	5.39	344.32	1.41	67.46	6.80	1.21	23.05	19.09	16	13.25
4(1)	2.41	6.77	85.33	106.85	21.15	53.40	4.85	340.52	3.20	79.70	7.59	0.81	18.76	23.14	9	11.10
254(2)	2.88	6.91	98.00	124.72	22.44	47.33	6.79	414.92	1.00	58.29	6.47	1.20	27.87	23.22	19	15.83
56(5)	3.08	7.91	84.40	122.12	21.44	41.29	6.19	99.12	1.42	41.38	2.10	1.22	20.47	16.78	10	8.20
149(3)	2.68	7.17	78.13	117.98	25.42	40.15	4.59	320.92	0.73	64.44	8.90	1.35	28.59	21.17	15	11.11
225(3)	3.01	10.24	94.40	93.52	20.22	35.99	9.12	849.02	2.38	88.00	17.82	1.42	39.05	27.51	17	11.98
127(4)	2.61	6.59	88.00	113.57	22.97	40.10	5.52	412.82	2.36	67.85	8.42	1.17	22.91	19.61	6	5.14
155(3)	2.68	6.71	83.40	104.12	20.71	37.35	6.39	380.32	1.36	70.30	7.32	1.58	21.31	13.50	20	12.67
16(1)	1.61	5.31	76.33	93.32	20.31	48.80	4.25	521.42	2.76	75.91	8.95	1.18	24.19	20.48	6	5.08
233(1)	3.01	6.77	81.53	100.05	26.68	53.28	5.65	219.92	3.20	42.67	5.45	1.13	19.92	17.65	15	13.29
82(4)	2.68	6.97	75.53	95.19	23.42	37.65	5.19	270.12	0.28	51.72	6.36	1.10	22.73	20.66	10	9.09
40(2)	2.28	3.51	75.60	109.32	23.11	39.83	3.59	187.12	1.63	61.44	4.20	1.13	20.23	17.93	5	4.43
113(3)	3.08	7.77	83.53	106.19	23.62	35.71	5.39	410.12	0.45	66.87	9.81	1.10	22.17	20.15	10	9.09
198(3)	3.08	5.11	88.80	142.52	24.77	46.93	4.39	529.32	1.14	64.06	8.94	1.50	31.93	21.30	25	16.68
110(4)	1.81	5.91	78.33	104.92	24.57	43.42	5.05	662.59	2.79	74.10	12.16	1.23	29.57	23.96	11	8.91
178(2)	2.61	9.24	89.20	117.52	25.15	39.13	7.52	504.62	2.29	70.96	9.95	1.42	26.11	18.39	7	4.93
74(3)	3.61	9.04	91.80	107.32	22.82	43.09	7.12	271.62	1.99	53.70	4.08	1.10	27.37	24.88	10	9.09
170(4)	2.41	6.91	77.33	84.72	20.97	34.70	4.45	371.62	2.44	62.98	4.90	1.02	18.89	18.47	8	7.82
11(3)	2.28	8.51	84.40	84.92	21.57	31.35	4.39	378.72	1.91	81.49	9.36	1.51	20.67	13.67	12	7.94
6(5)	2.68	5.91	74.53	99.59	18.88	714.60	1.99	224.12	0.19	68.26	5.24	1.10	14.00	12.73	10	9.09
218(1)	4.01	10.04	78.80	99.32	23.02	37.09	7.92	584.45	2.57	67.04	13.48	1.47	31.39	21.32	13	8.83

246(4)	2.21	6.37	96.13	132.05	25.42	55.20	7.45	423.12	2.84	49.95	7.59	1.38	33.52	24.30	12	8.70
44(1)	2.88	7.31	82.20	97.32	23.44	36.29	6.19	435.72	1.80	70.01	10.28	1.59	27.77	17.45	10	6.28
233(5)	3.01	7.91	82.23	91.62	23.07	40.20	5.45	270.52	2.43	53.92	3.04	1.17	20.48	17.53	13	11.13
220(4)	2.61	7.71	84.73	126.72	25.71	40.60	5.65	711.79	2.71	83.86	12.80	1.10	37.67	34.25	10	9.09
211(1)	2.28	4.51	75.00	103.32	19.44	38.41	4.19	448.92	1.17	69.70	7.75	0.88	20.89	23.82	5	5.70
243(2)	2.61	7.84	95.60	122.52	23.88	52.23	7.52	569.05	2.00	57.71	9.31	1.23	47.97	39.00	27	21.95
145(3)	2.68	5.77	77.53	117.58	22.62	41.65	3.79	247.52	0.23	58.87	5.76	1.02	22.91	22.41	11	10.76
52(4)	2.01	5.84	83.00	94.52	21.82	34.57	5.52	322.02	2.26	61.11	6.06	1.14	18.13	15.88	7	6.13
187(1)	3.01	9.04	95.40	87.12	31.35	48.41	8.92	697.45	2.17	72.62	12.93	1.10	35.59	32.30	5	4.54
195(2)	2.68	10.37	92.53	128.38	23.48	786.90	8.79	986.32	0.41	90.17	21.67	1.60	65.63	40.90	9	5.61
207(1)	2.61	8.04	79.80	116.52	21.55	33.67	6.72	330.82	2.49	64.32	7.23	1.32	20.65	15.64	9	6.82
164(1)	2.61	6.84	77.80	106.72	22.08	39.15	5.92	479.22	2.48	71.74	10.51	1.42	17.59	12.39	6	4.23
32(1)	2.61	6.84	95.60	102.32	22.62	47.81	5.92	412.22	2.32	66.75	8.21	1.47	30.83	20.94	9	6.11
137(4)	2.08	7.77	85.53	100.79	19.15	1925.50	5.79	535.72	0.25	77.79	11.37	0.97	24.41	25.18	7	7.22
204(1)	2.68	8.17	84.53	130.38	20.95	739.30	6.99	354.52	0.49	73.52	8.80	1.55	25.40	16.37	30	19.34
104(5)	2.11	8.59	92.25	102.82	21.47	32.90	6.27	122.32	2.62	55.49	2.85	1.10	22.83	20.75	10	9.09
236(3)	1.81	7.31	80.53	87.12	23.77	37.72	6.65	603.79	2.82	73.29	11.01	1.87	29.35	15.70	18	9.63
234(1)	2.08	7.57	98.13	135.58	24.48	48.35	6.19	701.92	0.06	78.61	13.13	1.87	37.17	19.89	12	6.42
64(5)	1.61	5.11	81.13	82.12	23.24	39.86	4.25	444.42	2.85	79.25	7.60	1.10	21.15	19.23	10	9.09
12(2)	1.68	5.57	74.53	102.25	18.99	694.90	3.32	448.12	0.13	78.28	9.10	1.10	17.13	15.57	10	9.09
50(3)	3.38	8.51	78.90	96.02	21.61	40.95	3.39	293.52	0.90	54.42	4.33	1.08	19.25	17.90	29	26.97
217(1)	1.68	6.57	97.53	134.78	22.48	807.30	5.79	593.12	0.15	74.91	11.84	1.42	32.67	23.02	17	11.98
255(5)	2.88	5.91	95.20	130.32	22.91	47.63	5.19	422.92	1.23	64.17	7.57	1.10	28.53	25.93	12	10.91
113(2)	2.01	6.91	85.93	102.12	23.17	35.30	6.65	448.02	3.17	56.33	8.73	1.10	29.25	26.59	13	11.82
119(4)	2.81	8.64	82.60	110.72	20.62	33.71	7.52	487.42	2.33	67.79	9.79	1.08	29.65	27.57	5	4.65
223(2)	2.81	8.84	95.40	109.12	25.62	42.35	6.52	627.85	2.19	72.33	11.75	1.10	32.37	29.42	17	15.45

38(3)	3.81	10.04	79.20	113.92	23.02	30.13	8.92	656.85	1.42	78.96	6.46	0.81	56.77	70.01	9	11.10
76(5)	2.81	8.04	87.60	116.32	21.75	43.41	7.32	408.62	2.29	77.71	7.96	1.10	29.21	26.55	14	12.73
213(4)	3.41	9.04	78.40	116.72	22.22	44.01	7.12	551.02	2.11	76.94	9.68	1.27	27.05	21.30	10	7.87
176(1)	2.61	9.31	87.33	118.32	24.31	43.72	7.65	910.82	2.82	79.16	17.81	1.56	45.81	29.37	22	14.10
216(1)	2.48	5.11	83.00	88.12	21.64	36.55	5.39	488.92	1.46	79.53	9.84	1.30	22.31	17.16	13	10.00
148(1)	1.95	9.17	95.33	100.32	21.44	42.25	8.52	923.22	2.14	81.34	16.96	1.56	39.47	25.30	19	12.18
38(3)*	2.61	6.91	81.33	104.52	22.37	41.12	5.65	484.62	2.44	63.58	7.00	1.55	25.31	16.31	7	4.51
178(2)	2.61	9.24	89.20	117.52	25.15	39.13	7.52	504.62	2.29	70.96	9.95	1.45	26.11	18.00	9	6.21
148(1)	1.95	9.17	95.33	100.32	21.44	42.25	8.52	923.22	2.14	81.34	16.96	1.41	39.47	28.07	36	25.60
208(4)	2.01	6.91	90.73	103.72	21.91	42.20	7.05	696.79	2.30	64.86	10.09	1.35	32.49	24.07	24	17.78
32(1)	2.61	6.84	95.60	102.32	22.62	47.81	5.92	412.22	2.32	66.75	8.21	1.47	30.83	20.94	9	6.11
211(3)	3.88	8.51	86.06	135.52	23.44	46.75	6.72	624.52	0.95	75.35	9.29	1.39	39.35	28.31	25	17.99
205(2)	4.88	10.71	79.80	114.92	20.77	40.97	6.39	233.92	1.48	54.07	4.84	1.44	24.19	16.80	13	9.03
96(4)	3.21	6.84	81.60	103.52	22.42	38.85	6.52	170.02	2.30	36.85	3.12	1.10	22.85	20.77	10	9.09
167(2)	2.61	8.04	78.80	94.12	20.02	26.39	5.92	345.02	2.38	73.63	7.08	1.06	14.09	13.29	7	6.60
147(1)	3.21	6.17	75.06	112.52	24.55	36.28	4.05	232.22	1.51	61.58	4.88	1.02	21.85	21.42	8	7.84
181(2)	2.01	7.84	91.80	116.72	23.95	39.47	5.72	563.85	2.31	68.12	11.29	1.32	30.21	22.88	19	14.39
103(5)	2.81	6.64	77.60	131.32	23.95	48.61	5.12	439.42	2.45	65.61	9.47	1.10	27.83	25.30	17	15.45
181(2)	2.01	7.84	91.80	116.72	23.95	39.47	5.72	563.85	2.31	68.12	11.29	1.53	30.21	19.74	23	15.03
75(2)	2.21	6.51	79.73	112.12	25.57	49.14	5.85	660.39	2.63	72.69	11.23	0.98	31.97	32.53	15	15.26
195(4)	3.01	9.11	89.33	107.12	22.64	41.84	7.85	784.82	2.55	81.45	13.26	0.99	36.09	36.46	16	16.16
138(2)	2.61	6.64	81.60	123.12	23.35	43.27	5.52	498.62	2.19	73.65	9.18	0.98	23.75	24.28	12	12.27
114(1)	2.81	11.04	95.60	98.92	23.95	55.13	8.52	811.22	2.47	80.50	17.84	1.42	44.15	31.09	29	20.42
242(4)	3.01	9.04	95.60	121.72	24.55	56.21	6.12	481.02	2.00	55.39	7.80	1.57	50.45	32.19	28	17.87
140(3)	2.61	6.24	80.40	117.92	23.42	38.35	5.72	206.42	2.02	62.37	3.03	0.98	17.55	17.91	6	6.12
240(2)	2.08	7.57	97.53	129.18	23.02	885.50	6.39	642.32	0.28	78.59	13.59	1.10	35.67	32.42	17	15.45

55(2)	1.81	5.97	88.33	119.65	21.02	47.44	5.05	120.32	3.08	33.28	3.35	1.23	19.16	15.58	11	8.94
57(5)	2.61	7.37	80.73	105.65	24.68	116.42	6.85	347.32	3.25	62.27	8.03	1.00	24.94	25.04	11	11.04
37(1)	2.68	5.91	77.20	86.32	21.31	31.09	6.59	418.52	1.45	69.97	8.39	1.10	23.03	20.94	10	9.09
156(2)	3.01	8.37	81.33	113.25	23.02	49.54	6.85	397.32	2.76	59.05	7.11	0.88	29.94	34.14	5	5.70
215(1)	2.01	5.31	80.13	125.32	24.37	37.28	4.65	476.42	2.92	66.69	8.56	1.10	32.97	29.97	15	13.64
248(2)	3.28	6.31	98.80	117.92	22.04	49.75	6.39	462.92	0.98	60.51	7.08	1.54	28.11	18.27	7	4.55
179(3)	2.81	7.31	87.13	112.12	24.04	45.66	6.25	799.22	2.73	84.48	14.75	1.11	37.95	34.19	22	19.82
161(1)	1.81	5.77	84.73	103.05	24.02	44.22	6.25	373.32	3.33	58.86	8.69	1.10	23.66	21.51	10	9.09
135(5)	2.81	8.42	99.08	93.45	23.30	42.85	7.40	367.02	3.35	55.26	9.00	1.00	26.21	26.21	13	13.00
76(1)	2.81	8.37	78.73	104.25	22.88	49.32	6.65	256.32	3.00	34.23	5.07	1.08	22.90	21.24	9	8.35
44(5)	3.01	7.71	75.73	97.92	21.77	33.44	5.65	360.22	2.87	48.15	5.74	1.37	23.83	17.44	30	21.96
103(2)	1.01	7.71	93.33	81.92	20.84	44.18	6.65	657.79	2.11	73.81	8.38	1.44	29.15	20.24	13	9.03
54(5)	3.21	9.37	88.53	118.85	23.95	55.30	8.05	594.12	2.90	68.22	10.59	1.43	39.90	27.85	31	21.64
17(3)	3.21	8.97	80.93	96.85	27.35	49.50	8.25	659.92	3.36	77.01	14.69	1.10	36.00	32.67	10	9.08
230(3)-1	6	13	74	115	21	28.8	5	244	2.2541	64.894	5.5	0.82	21.20	25.73	24.0	29.13
154(2)-1	4	9	74	85	25	39.0	7	586	3.0205	72.435	17.7	1.38	32.90	23.84	7.0	5.07
198(2)-1	2	11	73	123	24	40.8	9	819	1.7460	68.650	14.3	1.17	49.70	42.55	6.0	5.14
195(5)-3	4	12	86	117	22	45.0	12	1025	2.1854	83.333	22.4	1.63	53.50	32.80	11.0	6.74
132(2)-1	4	11	75	110	24	74.0	8	959	1.5328	71.728	14.7	1.22	38.30	31.37	36.0	29.48
61(1)-2	3	9	86	103	24	36.4	10	620	1.9516	61.876	12.1	1.27	36.40	28.57	7.0	5.49
18(2)-1	2	10	92	95	24	42.0	8	909	2.6513	92.472	24.1	1.54	43.50	28.28	29.0	18.86
80(1)-1	5	9	79	134	26	37.7	9	1146	1.7103	69.750	19.6	1.43	49.40	34.47	7.0	4.88
95(4)-1	5	8	79	115	26	51.0	8	820	1.5976	89.422	13.1	1.67	36.70	21.96	15.0	8.98
90(5)-5	3	9	85	110	25	37.2	8	698	2.2923	79.863	16	1.21	36.00	29.80	8.0	6.62
122(5)-1	4	9	82	110	26	46.4	9	487	2.0123	69.771	9.8	1.33	50.10	37.75	31.0	23.36
73(4)-1	4	14	98	109	25	48.0	13	1239	1.5335	67.853	19	0.94	57.70	61.19	19.0	20.15

8(4)-4	4	8	74	100	21	47.6	6	667	1.8591	78.563	12.4	0.75	28.50	38.26	14.0	18.79
153(4)-5	5	12	84	127	23	52.2	11	767	2.1512	45.682	16.5	1.58	54.20	34.35	28.0	17.74
206(5)-5	4	12	85	121	29	49.3	9	873	1.9244	70.177	16.8	1.60	49.50	30.86	22.0	13.72
220(2)-4	4	8	86	121	23	39.2	9	492	2.0122	69.986	9.9	1.50	37.20	24.82	72.0	48.03
181(4)-2	4	7	87	125	21	40.5	6	518	2.0463	81.191	10.6	1.34	32.60	24.33	10.0	7.46
201(2)-2	3	11	86	111	24	64.8	10	783	1.8263	81.056	14.3	1.02	37.90	37.08	17.0	16.63
83(4)-2	7	17	83	120	24	49.3	11	1048	1.9084	60.230	20	1.34	44.70	33.36	13.0	9.70
87(4)-2	4	11	88	175	22	36.0	12	1073	1.6775	87.024	18	1.34	66.50	49.63	5.0	3.73
222(3)-5	5	10	79	145	26	52.7	8	604	2.2020	84.594	13.3	0.81	47.70	58.82	36.0	44.39
33(1)-1	6	11	77	86	23	28.8	6	566	2.2438	71.555	12.7	1.30	30.40	23.38	15.0	11.54
188(4)-5	5	13	88	108	26	62.7	11	910	2.7473	74.286	25	1.17	54.30	46.49	15.0	12.84
102(4)-4	5	10	74	127	23	56.0	4	461	1.8221	92.757	8.4	0.84	25.20	30.11	34.0	40.62
17(3)-4	5	11	74	94	26	45.0	10	974	2.1869	77.240	21.3	1.10	50.10	45.46	18.0	16.33
11(4)-1	5	16	83	85	20	26.0	13	570	2.3509	85.329	13.4	1.01	27.20	26.96	11.0	10.90
34(3)-4	7	15	87	140	27	52.8	14	1323	1.8594	76.874	24.6	1.49	95.10	64.04	44.0	29.63
152(5)-1	6	18	76	124	26	44.8	12	1420	2.5000	92.508	35.5	1.00	87.80	88.15	31.0	31.12
122(5)-5	5	10	82	96	25	54.0	7	831	1.8893	73.670	15.7	1.33	47.10	35.49	31.0	23.36
215(2)-4	7	15	80	127	23	45.9	14	1141	2.1735	90.412	24.8	0.75	91.00	122.15	16.0	21.48
229(4)-3	8	23	99	132	25	26.0	20	2955	2.1895	91.204	64.7	1.45	151.90	105.05	41.0	28.35
172(3)-3	5	10	82	145	26	61.2	6	697	2.2525	74.386	15.7	1.14	48.50	42.49	34.0	29.78
59(4)-4	5	9	83	115	21	37.5	9	767	2.2816	85.698	17.5	1.70	43.20	25.46	11.0	6.48
22(2)-4	6	12	99	140	27	51.3	9	865	1.9075	73.995	16.5	1.45	69.00	47.72	47.0	32.50
163(1)-5	5	11	74	91	24	39.0	10	1022	2.1722	67.325	22.2	0.97	49.60	51.13	7.0	7.22
114(2)-4	5	13	87	120	30	58.9	8	1078	1.8924	78.400	20.4	1.83	53.60	29.31	33.0	18.04
81(4)-3	3	10	88	116	21	48.0	8	508	2.0079	80.892	10.2	1.34	32.30	24.11	34.0	25.37
193(1)-1	5	15	99	145	28	58.9	13	1873	2.3011	88.642	43.1	1.39	110.00	78.97	34.0	24.41
34(3)-1	5	12	93	142	26	55.8	9	1340	2.0373	82.360	27.3	1.49	81.30	54.75	44.0	29.63

65(3)-5	5	12	85	136	22	43.4	12	1145	1.8690	84.815	21.4	1.26	49.50	39.25	13.0	10.31
161(3)-1	2	7	85	120	21.67	29.9	7	364	2.1154	53.06	7.7	1.42	28.4	20.01	13.0	9.16
87(3)-4	2	7	102	125	22.00	48	5	156	1.4103	52.70	2.2	1.76	25	14.18	8.0	4.54
120(4)-2	2	5	84	87	20.33	37.8	4	129	1.9380	29.38	2.5	1.17	9.50	8.13	5.0	4.28
29(1)-3	5	10	78	105	23.00	50.4	7	12	1.6667	1.35	0.2	1.26	37.20	29.51	27.0	21.42
60(3)-4	3	8	89	95	22.67	35.1	6	138	1.8841	35.38	2.6	1.70	16.4	9.66	13.0	7.66
90(1)-2	2	7	92	125	21.67	16.5	3	67	1.9403	72.04	1.3	1.47	7.60	5.16	7.0	4.76
62(4)-4	2	6	78	87	21.00	33.8	6	145	1.6552	41.08	2.4	1.68	17	10.10	5.0	2.97
156(4)-2	2	6	84	96	22.33	36.4	4	156	1.6667	61.42	2.6	1.34	15.10	11.27	8.0	5.97
37(4)-5	1	4	79	106	26.33	49.6	4	465	1.5054	63.44	7	0.97	23.40	24.12	13.0	13.40
49(1)-3	3	8	99	124	22.67	55.1	8	46	1.3043	7.55	0.6	1.33	41.80	31.50	12.0	9.04
51(4)-3	3	9	74	110	19.67	42.0	3	52	2.3077	31.33	1.2	1.30	7.60	5.85	21.0	16.15
23(3)-3	3	6	90	117	24.67	36.4	6	259	1.8919	57.94	4.9	1.12	25.40	22.78	22.0	19.73
48(3)-2	2	4	83	105	21.33	42.5	4	148	1.6216	52.30	2.4	1.55	14.10	9.09	7.0	4.51
168(4)-3	3	7	86	116	24.67	45.5	6	212	1.5094	40.38	3.2	1.41	24.50	17.43	14.0	9.96
55(2)-3	2	6	92	136	24.33	49.3	6	245	1.7551	60.95	4.3	1.96	32.70	16.67	10.0	5.10
7(3)-1	2	6	77	100	19.67	34.8	3	233	1.7597	73.27	4.1	1.51	11.20	7.41	5.0	3.31
235(1)-2	3	6	97	90	17.00	31.5	6	221	0.9955	41.46	2.2	1.55	14.50	9.34	5.0	3.22
205(4)-2	2	6	74	124	27.67	45.9	6	386	1.9171	44.47	7.4	1.21	32.10	26.57	28.0	23.18
36(4)-3	3	7	78	122	19.67	41.6	7	176	1.8750	58.86	3.3	1.51	25.40	16.80	13.0	8.60
196(4)-1	2	6	82	100	24.33	46.4	5	281	1.3167	47.39	3.7	1.09	18	16.53	5.0	4.59
19(4)-1	3	8	79	90	11.67	52.7	2	112	1.3393	56.00	1.5	1.60	10.10	6.30	4.0	2.49
50(4)-1	3	6	74	101	8.00	40.3	1	95	2.0000	51.08	1.9	1.58	14.30	9.06	7.0	4.44
117(5)-1	2	5	79	119	21.67	43.5	5	318	1.7610	69.43	5.6	1.46	24.90	17.07	13.0	8.91
41(5)-5	3	6	82	110	24.33	43.5	4	126	1.9048	64.95	2.4	1.67	12.50	7.48	22.0	13.17

224(1)-3	2	4	74	97	16.67	35.0	3	251	1.7530	58.24	4.4	1.54	21.60	14.04	16.0	10.40
180(2)-1	3	7	79	105	23.00	48.0	5	211	1.9431	30.45	4.1	1.57	24.50	15.65	11.0	7.03
171(2)-1	3	9	79	95	22.33	55.8	8	130	1.5385	27.08	2	1.08	27.20	25.30	16.0	14.88
42(3)-2	1	4	83	116	28.67	60.0	5	130	1.3846	20.16	1.8	0.81	27.20	33.54	25.0	30.83
196(5)-1	2	8	97	140	25.33	50.0	7	186	1.8280	46.97	3.4	1.47	43.00	29.21	11.0	7.47
2(4)-3	3	6	74	96	13.67	42.0	2	161	1.9255	48.49	3.1	1.29	9.60	7.46	14.0	10.88
165(2)-4	1	5	84	104	22.00	36.4	5	158	1.6456	29.04	2.6	1.26	18.90	14.99	10.0	7.93
93(1)-2	2	5	74	100	16.00	41.6	2	211	1.5166	68.28	3.2	1.80	12.00	6.66	10.0	5.55
124(1)-1	2	7	84	142	23.67	56.1	7	321	1.8069	48.49	5.8	1.38	29.50	21.38	34.0	24.64
123(1)-2	2	5	74	108	23.67	58.8	5	298	1.7114	65.78	5.1	1.74	22.50	12.95	9.0	5.18
221(1)-5	1	4	74	95	22.33	45.5	4	205	1.7073	79.77	3.5	1.12	12.20	10.94	9.0	8.07
78(1)-1	2	8	81	90	21.67	36.4	7	221	1.6290	39.25	3.6	1.26	17.50	13.88	6.0	4.76
170(1)-5	2	6	84	94	22.00	32.5	4	147	2.3810	31.82	3.5	1.17	12.10	10.36	23.0	19.69
133(1)-1	4	9	85	135	24.00	46.2	4	88	1.7045	32.23	1.5	1.27	23.2	18.21	21.0	16.48
192(4)-4	2	7	80	121	21.67	46.4	5	166	1.2048	24.20	2	1.23	26.60	21.56	9.0	7.29
46(3)-2	2	8	83	103	23.33	39.0	7	155	1.6129	48.59	2.5	1.45	19.80	13.69	7.0	4.84

VITAE

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Academic Qualification

2006	Passed H.S.L.C examination with second division from Government Senior Secondary School, Temi, South Sikkim
2008	Passed Intermediate examination with second division from Government Senior Secondary School, Temi, South Sikkim
2012	Passed B.Sc. Agri. From college of agriculture, CAU, Imphal
2015	Passed M.Sc. (Agri.) Plant molecular biology and biotechnology from college of post graduate studies, CAU (Imphal), Recipient of CAU merit Scholarship

Achivements

Awarded National Eligibility Test (NET) in Plant Biotechnology (2015, 2016, 2018)
Awarded National Eligibility Test (NET)/Junior Research Fellowship (JRF) in Life Sciences (2015 and 2016)
Awarded Best Poster at National Conference on Plant Breeding in Genomics Era, UAS, Bengaluru
Awarded Best Paper award for a review paper titled "Use of Sequence Specific Nucleases for Site Specific Modification of Plant Genome for Crop Improvement
Awarded National Fellowship for Higher Education of ST Students (NFST)

Work experience

Worked as Horticulture Teaching Faculty at Government Senior Secondary School, Namthang, South Sikkim, HRDD. Govt. of Sikkim from July 2012 to Nov 2012

Publications

Use of sequence specific nucleases for site specific modification of plant genome for crop improvement
Mapping and Deployment of Blast Resistance Gene in Rice - A Work in Progress
In vitro direct regeneration of Dalle Khursani (*Capsicum annum*) from salicylic acid treated explants.
In vitro regeneration of Dalle Khursani, an important Chilli cultivar of Sikkim, using various explants
Effects of climate change on growth and development of chilli
Rich genetic diversity of *Capsicum species* in northeast India, as a potential source for chilli crop improvement.