Differential Expression Analysis of Phytic Acid Biosynthetic Genes in Aromatic and Non-Aromatic Rice Varieties of Kerala

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CERTIFICATE

This is to certify that the dissertation entitled "**Differential Expression Analysis of Phytic Acid Biosynthetic Genes in Aromatic and Non-Aromatic Rice Varieties of Kerala**" submitted to the University of Kerala in partial fulfilment of the requirements for the award of the degree of Master of Science in Biochemistry, is a record of original research work carried out by **THASBEERA N. (Candidate code: 63824142011)** of the Department of Biochemistry, T.K.M. College of Arts and Science, Kollam under the guidance and supervision of **Prof. (Dr.) R. Rajalakshmi & Dr. Nadiya F.,** in the Inter University Centre for Genomics and Gene Technology (IU-CGGT), University of Kerala, Kariavattom. It is also certified that no part of this work has been presented for the award of any other degree, diploma, or similar title at any other university.

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Declaration

I hereby declare that the thesis titled **"Differential Expression Analysis of Phytic Acid Biosynthetic Genes in Aromatic and Non-Aromatic Rice Varieties of Kerala"** is based on original research work carried out by me under the supervision of **Prof. (Dr.) R. Rajalakshmi** and **Dr. Nadiya F.,** in the Inter University Centre for Genomics and Gene Technology (IUCGGT), University of Kerala. I further declare that no part of this work has been presented for the award of any other degree, diploma, or similar title from any other university.

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Abbreviations

AGE	: Agarose Gel Electrophoresis
cDNA	: Complementary DNA
Ct	: Cycle Threshold
DAG	: Diacylglycerol
DEPC	: Diethyl Pyrocarbonate
EtBr	: Ethidium Bromide
IMP	: Inositol Monophosphatase
Ins3P	: *myo*-Inositol 3-Phosphate
IP_1	: Inositol Monophosphate
IP_2	: Inositol Bisphosphate
IP_3	: Inositol Trisphosphate
IP_4	: Inositol Tetrakisphosphate
IP_5	: Inositol Pentakisphosphate
IP_6	: Inositol Hexakisphosphate (Phytic Acid)
IPK	: Inositol Phosphate Kinase
ITPK	: Inositol Trisphosphate Kinase
MIK	: Myo-Inositol Kinase
MIPS	: Myo-Inositol-1-Phosphate Synthase
PA	: Phytic Acid
PCR	: Polymerase Chain Reaction
PLC	: Phospholipase C
PtdIns	: Phosphatidylinositol
PtdI4K	: Phosphatidylinositol 4-Kinase
qRT-PCR	: Quantitative Real-Time Polymerase Chain Reaction
RINO1	: Rice Myo-Inositol-1-Phosphate Synthase 1
RNA	: Ribonucleic Acid
TAE	: Tris–Acetate–EDTA

Abstract

Phytic acid is the major storage form of phosphorus in rice grains and plays an important role in seed development and mineral storage. However, its strong chelating property reduces the bioavailability of essential minerals such as iron, zinc, calcium, and magnesium. The present study investigated the expression of five key genes involved in phytic acid biosynthesis and transport (OsPGK1, RINO1, OsMIK, OsITPK1, and OsMRP13) in selected aromatic (Jeerakasala and Basmati) and non-aromatic released (Uma and Pratyasa) rice varieties of Kerala using quantitative real-time PCR (qRT-PCR).

High-quality RNA was isolated from young leaf tissues, followed by cDNA synthesis and qRT-PCR analysis using β-actin as the reference gene. Relative gene expression was calculated using the 2^-ΔΔCt method with Uma as the calibrator. Significant genotype-dependent variation was observed in the expression of all five genes. Jeerakasala showed the highest expression of OsPGK1, OsMIK, OsITPK1, and OsMRP13, while Basmati exhibited the highest expression of RINO1. Pratyasa showed comparatively lower expression of most genes, whereas Uma maintained baseline expression levels.

The study demonstrates that phytic acid biosynthesis is regulated through genotype-specific transcriptional mechanisms rather than by aromatic or non-aromatic classification alone. The observed variation among the rice varieties provides valuable molecular information for breeding programmes aimed at optimizing phytic acid content, improving mineral bioavailability, and enhancing the nutritional quality of rice. These findings also provide a basis for future studies on grain phytic acid content and the development of nutritionally improved rice cultivars.

Chapter 1

Introduction

Rice (*Oryza sativa* L.) is one of the most important cereal crops in the world and serves as the primary staple food for nearly half of the global population. Belonging to the family Poaceae, rice has played a crucial role in the culture, economy, and food security of billions of people for centuries. Owing to its significance in sustaining global nutrition, the United Nations declared 2004 as the "International Year of Rice" (Vanishri, 2016). Rice is an excellent source of carbohydrates and provides essential nutrients such as vitamins, minerals, dietary fiber, and bioactive compounds. In addition to its nutritional value, rice grains contain several phytochemicals, including phenolic compounds, flavonoids, tocopherols, tocotrienols, γ-oryzanol, and phytic acid, which contribute to their antioxidant and health-promoting properties (Mohidem et al., 2022).

Rice varieties are broadly classified into aromatic and non-aromatic types based on the presence or absence of characteristic fragrance. Aromatic rice varieties possess a unique aroma primarily attributed to the compound 2-acetyl-1-pyrroline (2-AP), which is highly valued by consumers and contributes significantly to market preference and economic value (Bhattacharjee et al., 2002). India is recognized as one of the richest areas of aromatic rice diversity, with numerous traditional cultivars possessing distinct grain quality traits (Bisne and Sarawgi, 2008). Among these, Jeerakasala and Basmati are renowned aromatic rice varieties known for their superior grain quality, cooking characteristics, and consumer acceptance. In contrast, non-aromatic varieties such as Prathyasa and Uma are widely cultivated because of their high yield potential, adaptability, and agronomic performance. Besides starch, rice grains contain several bioactive compounds and storage molecules that influence their nutritional quality. Among these, phytic acid (myo-inositol hexakisphosphate, IP_6) is the primary storage form of phosphorus in cereal grains, accounting for approximately 65–85% of the total seed phosphorus. The biochemical and genetic differences between aromatic and non-aromatic rice varieties may influence the accumulation of nutritionally important compounds, including phytic acid. Phytic acid plays an essential role in seed development, germination, and phosphorus storage; however, it is also considered an anti-nutritional factor because of its strong chelating ability toward mineral elements such as iron, zinc,

calcium, and magnesium. These mineral-phytate complexes are poorly absorbed in the human gastrointestinal tract, thereby reducing mineral bioavailability and contributing to micronutrient deficiencies in populations dependent on cereal-based diets.

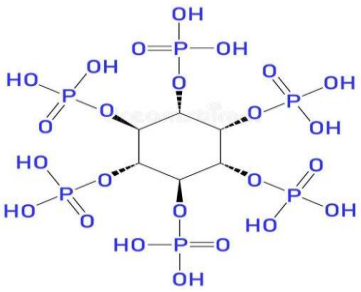
Chemical Structure of Phytic acid

Phytic acid consists of a myo-inositol ring, a six-carbon cyclic alcohol (cyclohexanehexol), in which each carbon atom bears a hydroxyl (-OH) group. In phytic acid, all six hydroxyl groups are esterified with phosphate groups, resulting in the formation of myo-inositol 1,2,3,4,5,6-hexakisphosphate (IP_6).

- Molecular Formula: $C_6H_{18}O_{24}P_6$
- Molecular Weight: 660.04 g/mol
- IUPAC Name: myo-Inositol 1,2,3,4,5,6-hexakis(dihydrogen phosphate)

The molecule contains:

- One six-membered myo-inositol ring
- Six phosphate ester groups
- Twelve ionizable hydrogen atoms associated with the phosphate groups



Structure of Phytic acid

Because of the presence of six phosphate groups, phytic acid possesses a high negative charge density at physiological pH, enabling it to strongly bind positively charged mineral ions.

The biosynthesis of phytic acid in plants occurs through a series of phosphorylation reactions involving myo-inositol intermediates. In rice, both lipid-dependent and lipid-independent pathways contribute to phytic acid formation, with the latter being predominant during seed

development. Several genes involved in phytic acid biosynthesis and accumulation have been identified, including RINO1 (Rice Inositol-3-Phosphate Synthase 1), INO1/MIPS, ITPK1, ITPK2, ITPK6, IPK1, MIK, LPA1, and MRP transporters. These genes regulate sequential steps leading to the synthesis and storage of phytic acid in developing grains. Among them, RINO1/INO1 encodes myo-inositol-3-phosphate synthase, which catalyses the first committed step in the pathway and is considered a key regulatory gene controlling phytic acid accumulation.

Phytic acid is synthesized through a series of phosphorylation reactions beginning with glucose-6-phosphate. In plants, the lipid-independent pathway is considered the major route for phytic acid accumulation in developing seeds, including rice grains.

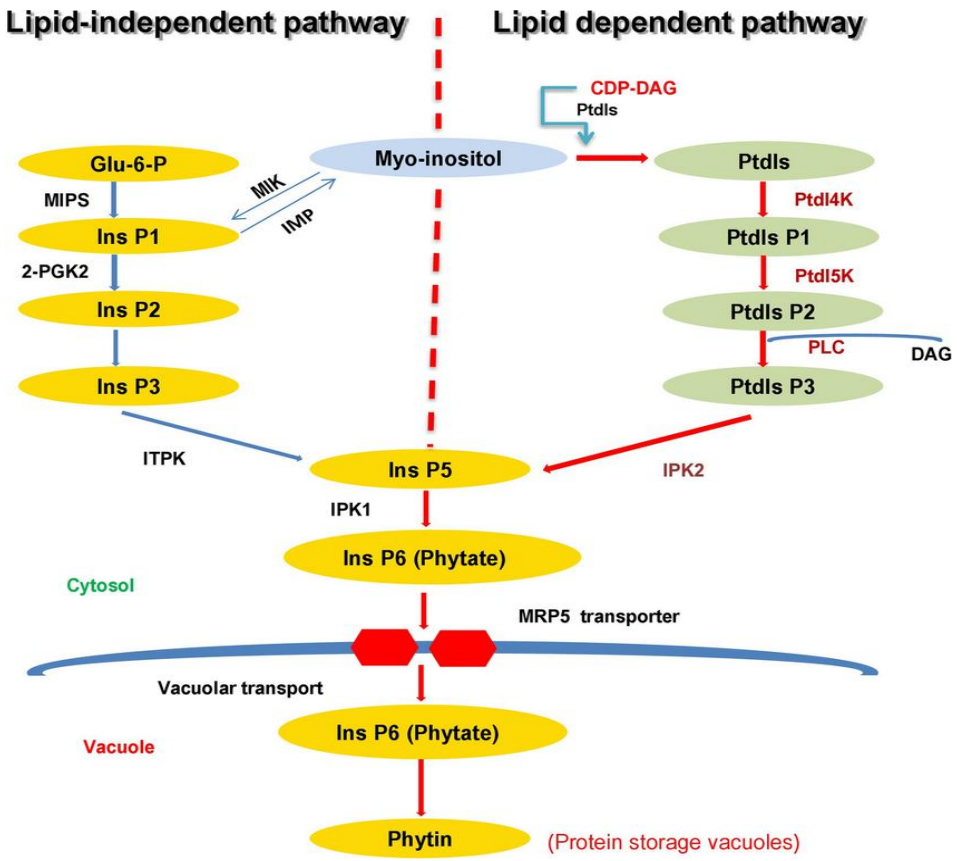


Figure 1: Phytic acid biosynthesis pathways (lipid independent pathway and lipid dependent pathway) (Kumar et al., 2023)

In plants, biosynthesis of phytic acid occurs through two pathways: the lipid-dependent pathway and the lipid-independent pathway. The lipid-dependent pathway operates in most plant tissues, whereas the lipid-independent pathway is predominant in developing seeds (Sparvoli and Cominelli,2015). The synthesis of phytic acid begins with the production of myo-inositol (Ins)

from glucose-6-phosphate. This reaction is catalysed by the enzyme myo-inositol-3-phosphate synthase (MIPS), which converts glucose-6-phosphate into myo-inositol-3-phosphate [Ins(3)P_1]. Subsequently, inositol monophosphatase (IMP) dephosphorylates Ins (3)P_1 to produce free myo-inositol. Besides its role in phytic acid synthesis, IMP also participates in ascorbic acid (vitamin C) biosynthesis by hydrolyzing l-galactose-1-phosphate. (Francesca Sparvoli and Eleonora Cominelli,2015). The free myo-inositol is then phosphorylated by myo-inositol kinase (MIK) to form inositol monophosphate intermediates. Although myo-inositol-3-phosphate can be directly produced by MIPS, MIK is considered important because it generates multiple inositol phosphate intermediates that contribute to phytic acid accumulation in seeds. (Francesca Sparvoli and Eleonora Cominelli,2015).

In the lipid-dependent pathway, myo-inositol is first converted into phosphatidylinositol (PtdIns) by phosphatidylinositol synthase. The phosphatidylinositol molecule is then phosphorylated by phosphatidylinositol kinases to form PtdIns (4,5) P_2, which is subsequently hydrolysed by phospholipase C to release inositol 1,4,5-trisphosphate [Ins (1,4,5) P_3], an important signalling molecule involved in cellular signal transduction. In contrast, the lipid-independent pathway does not involve phosphatidylinositol synthesis. Instead, it proceeds through the sequential phosphorylation of myo-inositol and its intermediates to form phytic acid ($InsP_6$). After the formation of $InsP_1$ by MIK, the conversion of $InsP_1$ to $InsP_2$ is believed to be catalysed by a 2-phosphoglycerate kinase homolog (2-PGK/OsPGK1). Studies in rice have shown that mutations in OsPGK1 reduce phytic acid accumulation, whereas overexpression of this gene increases seed phytic acid content, indicating that OsPGK1 may catalyze a rate-limiting step in phytic acid biosynthesis. (Sparvoli andCominelli,2015). Further phosphorylation steps convert $InsP_3$ into $InsP_4$, $InsP_5$, and finally $InsP_6$. These reactions are catalysed by three major classes of inositol phosphate kinases: inositol polyphosphate multikinase (IPK2/IPMK), inositol 1,3,4-trisphosphate 5/6-kinase (ITPK), and inositol polyphosphate 2-kinase (IPK1). Among these, IPK1 catalyzes the final phosphorylation step, converting inositol pentakisphosphate ($InsP_5$) into phytic acid ($InsP_6$).Thus, the coordinated action of MIPS, IMP, MIK, OsPGK1, IPK2, ITPK, and IPK1 leads to the synthesis and accumulation of phytic acid in rice seeds, where it serves as the principal storage form of phosphorus (Sparvoli and Cominelli,2015).

Once phytic acid ($InsP_6$) is synthesized, it is stored in specialized structures called globoids within the protein storage vacuoles of developing seeds. The transport of phytic acid into these vacuoles is mediated by a specific $InsP_6$ transporter, known as the multidrug resistance-associated protein (MRP), which belongs to the ATP-binding cassette (ABC) transporter family. This active transport process is essential for the accumulation and storage of phytic acid in seeds. The roles of the enzymes and transport proteins involved in phytic acid synthesis and storage have been confirmed through enzyme isolation, biochemical characterization, and studies on low phytic acid (lpa) mutants, in which mutations in these genes result in reduced phytic acid accumulation (Torabinejad et al,2009). Several genes participate in this pathway by encoding enzymes responsible for the synthesis, phosphorylation, and transport of phytic acid in developing rice seeds. Among these, OsPGK1, RINO1, OsMIK, OsITPK1, and OsMRP13 play important roles.

OsPGK1 (Oryza sativa 2-Phosphoglycerate Kinase 1): OsPGK1, also known as the Lpa1 gene, encodes a protein homologous to 2-phosphoglycerate kinase. It is believed to function as an inositol monophosphate kinase, catalysing the conversion of inositol monophosphate ($InsP_1$) to inositol diphosphate ($InsP_2$), an important step in the phytic acid biosynthetic pathway. This step represents a major branch point between general inositol metabolism and phytic acid synthesis. Studies have shown that overexpression of OsPGK1 significantly increases phytic acid and total phosphorus accumulation in rice seeds, whereas mutations in this gene result in a low-phytic-acid phenotype. Therefore, OsPGK1 is considered a key regulatory gene controlling phytic acid accumulation in developing rice grains (Kim et al.,2008; Zhao et al., 2008).

RINO1 (Rice Myo-Inositol-1-Phosphate Synthase 1): RINO1 encodes myo-inositol-1-phosphate synthase (MIPS), the enzyme responsible for the first committed step in phytic acid biosynthesis. It catalyzes the conversion of glucose-6-phosphate into myo-inositol-1-phosphate, which serves as the precursor for all subsequent inositol phosphate intermediates. RINO1 is highly expressed in developing rice seeds, particularly in the embryo and aleurone layer, indicating its crucial role in phytic acid production. Because it initiates the biosynthetic pathway, RINO1 is often regarded as one of the most important genes regulating phytic acid synthesis in rice ((Tagashira et al., 2015; Perera et al., 2018).

OsMIK (Oryza sativa Myo-Inositol Kinase): OsMIK encodes myo-inositol kinase, an enzyme that phosphorylates free myo-inositol to generate phosphorylated inositol intermediates. These

intermediates undergo further phosphorylation reactions to ultimately produce phytic acid ($InsP_6$). OsMIK therefore plays an important role in maintaining the flow of metabolites through the phytic acid biosynthetic pathway. Increased expression of OsMIK has been associated with enhanced activity of phytic acid biosynthesis in rice tissues (Perera et al., 2018).

OsITPK1 (Oryza sativa Inositol 1,3,4-Trisphosphate 5/6-Kinase 1): OsITPK1 encodes inositol 1,3,4-trisphosphate 5/6-kinase (ITPK), an enzyme involved in the sequential phosphorylation of lower inositol phosphates into higher phosphorylated intermediates. It catalyzes important steps leading to the formation of inositol pentakisphosphate ($InsP_5$), which is subsequently converted into phytic acid ($InsP_6$). Studies have demonstrated that down-regulation of OsITPK1 significantly reduces phytic acid accumulation in rice seeds, indicating its critical role in determining grain phytic acid content (Karmakar et al., 2020).

OsMRP13 (Oryza sativa Multidrug Resistance-Associated Protein 13/OsABCC13): OsMRP13, also known as OsABCC13, does not directly synthesize phytic acid. Instead, it functions as a putative phytic acid transporter. The gene encodes an ATP-binding cassette (ABC) transporter that is believed to transport phytic acid ($InsP_6$) into storage vacuoles of developing rice seeds. Proper transport and compartmentalization of phytic acid are essential for its accumulation in grains. Therefore, OsMRP13 plays an indirect but important role in phytic acid metabolism by regulating the storage and deposition of phytic acid in rice seeds (Perera et al., 2018).

Phytic acid (PA), chemically known as myo-inositol-1,2,3,4,5,6-hexakisphosphate (IP_6), is the principal storage form of phosphorus in seeds and cereal grains. It accounts for approximately 1–7% of seed dry weight and may constitute up to 85% of the total phosphorus present in rice grains (Raboy, 1997). Structurally, phytic acid consists of a myo-inositol ring esterified with six phosphate groups, resulting in a highly negatively charged molecule with a strong affinity for multivalent mineral cations such as iron, zinc, calcium, magnesium, and potassium. During seed germination, phytate reserves are hydrolysed by phytase enzymes, releasing phosphorus, minerals, and myo-inositol necessary for seedling development (Afify et al., 2011).

Phytic acid plays a vital physiological role in plants by serving as a phosphorus reserve, regulating cellular signaling pathways, and contributing to seed development and stress responses. Rice plants continuously accumulate phosphorus throughout their growth cycle, with phosphorus initially stored in vegetative tissues before being remobilized and transported to developing grains

during maturation (Rose et al., 2010). The phosphorus deposited in grains originates from both post-flowering uptake and remobilization from vegetative tissues, where it is subsequently converted into phytic acid (Julia et al., 2016). This process highlights the close relationship between phosphorus metabolism and phytic acid accumulation in rice grains. Despite its essential role in plant physiology, phytic acid is widely regarded as an anti-nutritional factor because of its strong mineral-chelating properties. The six phosphate groups present in phytic acid readily bind mineral ions, forming insoluble phytate complexes that are poorly absorbed in the human gastrointestinal tract. Humans and other monogastric animals possess little or no endogenous phytase activity and are therefore unable to efficiently hydrolyse phytate during digestion (Wilson et al., 2015). Consequently, minerals bound to phytate remain unavailable for absorption, reducing the bioavailability of essential micronutrients such as iron and zinc. This issue is particularly important in populations that rely heavily on cereal-based diets, where high phytate consumption has been associated with micronutrient deficiencies and malnutrition (Al Hasan et al., 2016).

Iron and zinc deficiencies remain among the most widespread nutritional disorders worldwide. Iron is essential for haemoglobin synthesis, oxygen transport, energy metabolism, and cognitive development, whereas zinc functions as a cofactor for over 300 enzymes involved in growth, immune responses, and cellular metabolism (McCall et al., 2000). Phytic acid significantly reduces the absorption of these minerals by forming stable phytate-mineral complexes within the digestive tract (Couzy et al., 1998). The adverse effects of phytate are particularly pronounced among infants, pregnant women, and lactating mothers who depend largely on cereal-based diets for their nutritional requirements (Chan et al., 2007; Al Hasan et al., 2016). Therefore, reducing phytic acid accumulation in cereal grains has become a major target for nutritional improvement programs.

In addition to mineral chelation, phytic acid can interact with proteins, amino acids, digestive enzymes, and carbohydrates, thereby influencing nutrient digestibility and metabolism (Wang and Guo, 2021). It inhibits the activity of α-amylase, an enzyme involved in starch digestion, and may consequently affect starch hydrolysis and glycemic response. Increased phytic acid content has been associated with a reduction in the glycemic index of certain foods, suggesting a possible role in glucose metabolism and diabetes management (Kumar et al., 2020). Although traditionally regarded as an anti-nutritional compound, phytic acid has also been shown to possess several beneficial biological functions. Its strong antioxidant activity enables it to inhibit free radical

formation and reduce lipid peroxidation through iron chelation. Studies have further suggested that phytic acid may contribute to the prevention of cardiovascular diseases, reduction of plasma cholesterol and triglyceride levels, suppression of cancer cell proliferation, modulation of glucose absorption, and prevention of kidney stone formation (Zhou and Erdman, 1995; Kumar et al., 2023). These findings indicate that phytic acid exerts both beneficial and detrimental effects depending on its concentration and dietary context.

The biosynthesis of phytic acid in plants occurs through a series of enzymatic phosphorylation reactions involving myo-inositol intermediates. The pathway begins with the conversion of glucose-6-phosphate into myo-inositol-3-phosphate by myo-inositol-3-phosphate synthase (MIPS), encoded by the RINO1 gene. This step is considered the first committed and rate-limiting step of phytic acid biosynthesis. Subsequent phosphorylation reactions mediated by inositol phosphate kinases, including inositol trisphosphate kinases (ITPKs) and inositol pentakisphosphate kinase (IPK1), ultimately lead to the formation of phytic acid (Suzuki et al., 2007). The expression of these genes plays a crucial role in determining the extent of phytate accumulation in developing rice grains.

Several studies have demonstrated that genetic variation in grain phytic acid content is associated with differential expression of phytic acid biosynthetic genes. Elevated expression of genes such as RINO1, ITPK, and IPK1 has been correlated with increased phytate accumulation, whereas mutations or reduced expression of these genes result in low-phytate phenotypes (Perera et al., 2019). Such low-phytate rice lines have attracted considerable attention because they improve mineral bioavailability and reduce phosphorus excretion into the environment. Consequently, understanding the regulation of phytic acid biosynthesis at the molecular level has become increasingly important for rice biofortification programs. Despite the nutritional and agricultural importance of phytic acid, information regarding the expression patterns of phytic acid biosynthetic genes in aromatic and non-aromatic rice varieties remains limited. Aromatic rice cultivars possess unique metabolic characteristics associated with grain quality and aroma development, which may influence phosphorus metabolism and phytate accumulation. Comparative evaluation of aromatic and non-aromatic rice varieties could therefore provide valuable insights into the genetic regulation of phytic acid biosynthesis. Recent advances in molecular biology and transcriptomics have revealed that phytic acid accumulation is controlled

by a complex network of genes involved in myo-inositol metabolism, phosphorylation pathways, phosphorus transport, and seed storage processes. Differential expression of these genes among rice genotypes contributes to variation in grain phytic acid content.

Therefore, the present study was undertaken to check the expression patterns of phytic acid biosynthetic genes in selected aromatic and non-aromatic rice varieties of Kerala. Jeerakasala and Basmati were selected as representative aromatic rice varieties, while Prathyasa and Uma were selected as non-aromatic varieties which are also released varieties form Rice Research Station (RRS), Kerala Agricultural University, Moncombu, Alappuzha. Quantitative real-time PCR (qRT-PCR) was employed to evaluate the relative expression levels of key genes involved in phytic acid biosynthesis. The study aims to identify variations in gene expression associated with phytate accumulation and to improve our understanding of the molecular mechanisms governing phosphorus storage and nutritional quality in rice grains. The findings are expected to contribute to the development of rice varieties with optimized phytic acid content, improved mineral bioavailability, and enhanced nutritional value.

Chapter 2

Review of Literature

Rice (*Oryza sativa* L.) is the staple food for more than half of the world's population and serves as a major source of dietary energy, especially in Asian countries. Besides yield and productivity, nutritional quality has become an important breeding objective due to widespread micronutrient deficiencies. Among the nutritional factors present in rice grains, phytic acid (PA) is of particular importance because it functions both as a phosphorus storage compound and an anti-nutritional factor (Perera et al., 2018). Phytic acid, chemically known as myo-inositol hexakisphosphate (IP_6), constitutes approximately 65–85% of the total phosphorus in rice grains and accumulates mainly in the aleurone layer and embryo (Perera et al., 2018). Although PA plays a critical role in seed development and germination, it chelates essential minerals such as iron, zinc, calcium, and magnesium, thereby reducing their bioavailability in humans (Azharudheen et al., 2022). Recent advances in genomics and transcriptomics have revealed that variation in grain PA content is controlled by differential expression of genes involved in myo-inositol biosynthesis, phosphorylation pathways, and phosphorus transport systems (Perera et al., 2018). Understanding these molecular mechanisms is essential for developing nutritionally improved rice varieties. Significant genetic variation exists for grain PA content among rice genotypes. Azharudheen et al. (2022) evaluated 96 rice varieties and reported a wide range of PA content varying from 0.30% to 2.98%. Their study further demonstrated that genotype × environment interactions significantly influence grain phytate accumulation, indicating polygenic inheritance of the trait. The authors identified functional alleles in transporter genes such as SPDT and OsPT8 that were significantly associated with natural variation in grain PA content (Azharudheen et al., 2022). Such variability provides opportunities for identifying naturally low-phytate genotypes suitable for breeding programs. Aromatic rice varieties such as Basmati, Jeerakasala, Gandhakasala, and Joha possess unique grain quality attributes and command premium market value. Aroma in rice is primarily controlled by mutations in the BADH2 gene, leading to accumulation of 2-acetyl-1-pyrroline (2AP) (Gong et al., 2023). In addition to aroma, aromatic rice varieties exhibit distinct nutritional characteristics including differences in mineral composition, phenolic content, antioxidant activity, and grain quality parameters (Gong et al., 2023).

Rice aroma is a complex trait influenced by the interaction of volatile organic compounds, genetic factors, cultivation practices, post-harvest processing, and storage conditions. Hu et al. (2020) reported that more than 300 volatile compounds have been identified in rice grains, among which 2-acetyl-1-pyrroline (2-AP) is recognized as the primary compound responsible for the characteristic aroma of fragrant rice varieties. In addition to 2-AP, aldehydes, alcohols, ketones, and heterocyclic compounds contribute significantly to aroma quality. The authors further highlighted that sensory evaluation, gas chromatography (GC), gas chromatography–olfactometry–mass spectrometry (GC-O-MS), and electronic nose (E-nose) technologies are widely employed for aroma characterization. Environmental factors, milling degree, roasting, parboiling, processing techniques, and storage conditions were also found to significantly influence aroma development and retention in rice grains (Hu et al., 2020).

Genetic variability among aromatic and non-aromatic rice varieties has been extensively investigated for yield and agronomic traits. Cheema et al. (2005) conducted association and path coefficient analyses to evaluate yield-related traits in aromatic and non-aromatic rice genotypes. Their results demonstrated substantial genetic diversity among the studied varieties. In non-aromatic rice, the number of productive tillers per hill showed the strongest positive association with grain yield. In contrast, spikelets per panicle, fertility percentage, and plant height were identified as major determinants of yield in aromatic rice varieties. The study emphasized that different breeding strategies may be required for aromatic and non-aromatic rice because of their distinct genetic relationships among agronomic traits (Cheema et al., 2005).

The nutritional quality of aromatic rice has attracted considerable research interest due to its potential health benefits. Ghosh and Roychoudhury (2020) evaluated eleven indigenous aromatic rice varieties for nutritional composition, antioxidant properties, and associated gene expression patterns in both polished and unpolished grains. Their findings revealed substantial genotypic variation in micronutrient content, antioxidant activity, and nutritional quality. Among the studied genotypes, Tulaipanji exhibited superior nutritional characteristics, including higher concentrations of iron, zinc, and copper, which corresponded with increased expression of *FER2*, *ZIP*, and *NAS3* genes. The variety also showed elevated levels of amino acids, phenolics, flavonoids, tocopherols, anthocyanins, and antioxidant capacity. Increased expression of *PAL*, *ANS*, *PPO*, *LOX1*, and *HGGT* genes was associated with these nutritional advantages.

Furthermore, the genotype IET-21261 accumulated higher levels of phytic acid and total phosphorus, which correlated with enhanced expression of the *IPK1* gene involved in phytic acid biosynthesis. The study demonstrated that polishing significantly reduced nutritional quality across all rice genotypes, emphasizing the nutritional superiority of unpolished aromatic rice grains (Ghosh & Roychoudhury, 2020).

Phytic acid (myo-inositol hexakisphosphate; IP6) is the primary storage form of phosphorus in seeds and cereal grains. According to Perera et al. (2018), phytic acid accounts for approximately 65–85% of total phosphorus in rice grains and plays a critical role in phosphorus storage during seed development and germination. Despite its physiological importance, phytic acid is widely recognized as an anti-nutritional factor because of its strong chelating capacity toward mineral ions such as iron, zinc, calcium, and magnesium. The resulting phytate–mineral complexes are poorly absorbed by the human digestive system, thereby reducing mineral bioavailability and contributing to micronutrient deficiencies in populations that rely heavily on cereal-based diets (Perera et al., 2018).

Graf (1983) described phytic acid as a multifunctional molecule with applications extending beyond nutrition. While its ability to chelate minerals can negatively affect nutrient absorption, phytic acid also exhibits antioxidant properties and has potential applications in medical, pharmaceutical, dental, and industrial sectors. Therefore, phytic acid should not be regarded solely as an anti-nutritional factor but rather as a biologically important compound with both beneficial and detrimental effects depending on context (Graf, 1983).

Cominelli et al. (2020) reported that phytic acid functions not only as a phosphorus reserve but also as a regulator of numerous cellular processes in plants. To improve mineral bioavailability, significant efforts have been directed toward the development of low-phytic acid (lpa) mutants. These mutants arise through alterations in genes involved in phytic acid biosynthesis or transport processes. Transporter genes encoding ATP-binding cassette (ABCC) proteins, sulfate transporters, and phosphate transporters have been identified as important targets for reducing phytic acid accumulation while maintaining seed viability. The authors suggested that transporter-based low-phytate mutants represent promising resources for crop biofortification programs aimed at enhancing mineral nutrition (Cominelli et al., 2020).

Kumar et al. (2020) investigated the relationship between phytic acid accumulation, starch digestibility, and glycemic index among diverse rice genotypes. The study demonstrated that rice varieties with lower phytic acid content exhibited higher α-amylase activity, resulting in enhanced starch digestibility and elevated glycemic index values. Conversely, varieties characterized by higher phytic acid concentrations showed reduced starch digestibility and lower glycemic responses. Furthermore, the expression levels of key phytic acid biosynthetic genes, namely *MIPS1* (*Myo-inositol-3-phosphate synthase 1*) and *IPK1* (*Inositol pentakisphosphate kinase 1*), were significantly upregulated during the middle stages of grain development in high-phytate rice genotypes. These findings suggest a negative correlation between phytic acid accumulation and glycemic index, indicating that increased phytate content may inhibit enzymatic starch hydrolysis in the gastrointestinal tract, thereby reducing postprandial glucose responses and contributing to a lower glycemic index.

Raboy (2020) emphasized the significance of low-phytic acid (lpa) crops as a sustainable approach for improving nutritional quality and agricultural productivity. The author reported that the low-phytate trait enhances phosphorus utilization efficiency and reduces phosphorus excretion in non-ruminant animals, including poultry, swine, and fish, thereby mitigating environmental pollution associated with phosphorus runoff. In addition, low-phytate crops improve the bioavailability of essential mineral nutrients such as iron, zinc, calcium, and magnesium for both humans and livestock. Although certain low-phytate genotypes may exhibit reduced agronomic performance and yield penalties, advances in plant breeding and genetic engineering have provided effective strategies for overcoming these limitations. Raboy (2020) concluded that the development of high-yielding, stress-tolerant low-phytate crop varieties could play a crucial role in addressing global micronutrient deficiencies while promoting sustainable agricultural systems.

Azharudheen et al. (2022) investigated the natural genetic variation in grain phytic acid content among diverse rice germplasm and developed functional markers associated with phytic acid-related genes. Their study revealed substantial genotypic variation in grain phytate concentration, indicating that genetic factors play a major role in determining phytic acid accumulation. The authors identified specific DNA markers significantly associated with grain phytic acid content, which can serve as valuable genomic resources for marker-assisted breeding programs aimed at developing rice varieties with reduced phytate levels and improved mineral bioavailability.

Desingu et al. (2025) reviewed current strategies for mitigating the adverse effects of phytic acid while enhancing phosphorus utilization efficiency in agricultural systems. The authors highlighted that phytase supplementation is commonly employed in non-ruminant animal nutrition to improve phosphorus availability; however, excessive phosphorus excretion resulting from phytase use may contribute to environmental issues such as eutrophication. Alternative approaches, including optimized fertilizer management, advanced food processing technologies, and the development of low-phytate crop varieties, were identified as sustainable solutions for improving phosphorus utilization and minimizing environmental impacts. The review further emphasized the need for a comprehensive understanding of the molecular and physiological mechanisms underlying phytic acid biosynthesis, grain distribution patterns, environmental influences on phytate accumulation, and strategies to enhance mineral bioavailability through phytate reduction.

Burbano et al. (1995), Azeke et al. (2011), and Hayakawa et al. (1990) reported that one of the major nutritional concerns associated with dietary phytic acid is its negative effect on mineral bioavailability. Phytic acid undergoes enzymatic hydrolysis by phytase enzymes, producing lower inositol phosphates including inositol pentaphosphate ($InsP_5$), inositol tetraphosphate ($InsP_4$), inositol triphosphate ($InsP_3$), inositol diphosphate ($InsP_2$), and inositol monophosphate ($InsP_1$). This degradation occurs during grain storage, fermentation, germination, food processing, and digestion within the gastrointestinal tract. However, Sandberg et al. (1999) demonstrated that $InsP_6$ and $InsP_5$ remain the principal forms responsible for inhibiting mineral absorption due to their strong chelating properties.

Miller, L.V. et al. (2007), Couzy et al. (1998), and Kies et al. (2006) highlighted the critical role of zinc in human health and the detrimental consequences of zinc deficiency, including impaired growth, compromised immune function, increased morbidity and mortality, and adverse effects on neurological development. Phytate is recognized as one of the primary dietary factors limiting zinc absorption because of its strong affinity for divalent cations. In addition to mineral chelation, phytate has been reported to interact with proteins and digestive enzymes, potentially interfering with proteolytic processes through alterations in protein structure and enzyme accessibility. Consequently, high dietary phytate levels may contribute not only to mineral deficiencies but also to reduced protein digestibility and overall nutrient utilization.

Chan et al. (2007) and Al Hasan et al. (2016) reported that the adverse nutritional effects of phytic acid are particularly pronounced among vulnerable population groups, including infants, pregnant women, and lactating mothers, whose diets rely heavily on cereal-based foods. Due to the strong chelating ability of phytic acid, the absorption of essential minerals such as iron, zinc, and calcium is significantly reduced, thereby increasing the risk of micronutrient deficiencies. Consequently, dietary strategies aimed at reducing phytic acid intake or enhancing phytate degradation are considered important for improving mineral bioavailability and nutritional status in these high-risk groups. Furthermore, reducing phytic acid levels in cereal grains has been recognized as a key approach for enhancing the nutritional quality of both human and animal diets.

Despite its negative effects on mineral absorption, phytic acid also performs several important physiological functions in plants. Doria et al. (2009) reported that phytic acid serves as a natural antioxidant and plays a crucial role in protecting seeds against oxidative damage during maturation, storage, and germination. Its antioxidant properties contribute to seed longevity and maintenance of cellular integrity under stress conditions.

Beyond its role in plants, phytic acid and its phosphorylated derivatives participate in numerous cellular processes in eukaryotic organisms. Saiardi et al. (2002) and Bolger et al. (2008) demonstrated that in yeast, inositol phosphates derived from phytic acid are involved in several essential cellular functions, including mRNA export, translation regulation, RNA editing, DNA repair mechanisms, and vesicular trafficking. These findings indicate that phytic acid functions not only as a phosphorus storage compound but also as an important signaling molecule involved in diverse biological processes.

Raboy (2000) and Bregitzer and Raboy (2006) emphasized that humans and other monogastric animals lack endogenous phytase activity, limiting their ability to hydrolyse phytate complexes and release bound mineral ions such as Fe^{2+}, Zn^{2+}, and Ca^{2+}. Consequently, diets rich in whole grains and legumes containing high levels of phytic acid may contribute to mineral deficiencies, particularly in populations dependent on cereal-based foods as staple dietary components. This limitation has stimulated considerable interest in developing low-phytate crop varieties to improve mineral bioavailability.

Studies by Liu et al. (2004) and Sakai et al. (2015) demonstrated that reductions in phytic acid content significantly influence mineral distribution within rice grains. In conventional rice seeds,

minerals such as phosphorus, sulfur, potassium, and calcium are predominantly localized in the embryo and aleurone layer, with relatively low concentrations in the endosperm. However, low-phytate (lpa) rice mutants exhibited increased accumulation of these minerals in the endosperm, the edible portion of the grain. This redistribution is attributed to the reduced formation of phytate–mineral complexes, which facilitates greater mineral mobility and deposition within the endosperm. Consequently, low-phytate rice varieties may enhance the nutritional availability of essential minerals for human consumption.

Perera et al. (2018) highlighted biofortification and phytic acid reduction as complementary strategies for improving micronutrient bioavailability in rice grains. By simultaneously increasing mineral concentrations and reducing phytate-mediated mineral chelation, these approaches can substantially enhance the nutritional quality of rice and contribute to alleviating micronutrient malnutrition in populations dependent on rice as a staple food.

Wang et al. (2011) reported substantial genotypic variation in mineral composition and phytic acid content among diverse rice germplasm, indicating significant opportunities for genetic improvement. Several traditional landraces and wild rice accessions possess elevated concentrations of iron and zinc in the grain endosperm, making them valuable genetic resources for biofortification programs. Previous studies by Cheng et al. (2005), Garcia-Oliveira et al. (2009), Sarla et al. (2012), and Mahender et al. (2016) identified numerous rice genotypes with enhanced micronutrient accumulation that can serve as donor parents in marker-assisted breeding programs. The identification and utilization of rice varieties combining high micronutrient concentrations with low phytic acid content are therefore considered promising strategies for combating iron and zinc deficiencies and improving grain nutritional quality.

Suzuki et al. (2007) investigated the expression profiles of genes involved in phytic acid biosynthesis during rice seed development and identified twelve genes associated with inositol phosphate metabolism, including *MIPS*, *IMP*, *IPK1*, *IPK2*, and *ITP5/6K*. The study revealed significant upregulation of *MIPS*, *IPK1*, and *ITP5/6K* transcripts during embryo development, suggesting that the lipid-independent pathway plays a predominant role in phytic acid biosynthesis during seed formation. Furthermore, the authors proposed that both lipid-dependent and lipid-independent pathways operate in rice anthers, indicating tissue-specific regulation of phytate biosynthesis.

Among the genes involved in phytic acid biosynthesis, RINO1 (*Rice Inositol-3-Phosphate Synthase 1*) plays a critical role. The RINO1 gene encodes myo-inositol-3-phosphate synthase, which catalyzes the first committed step in the biosynthetic pathway by converting glucose-6-phosphate into myo-inositol-3-phosphate (Yoshida et al., 1999). Expression analyses have demonstrated that RINO1 transcripts accumulate predominantly in developing embryos and aleurone tissues during grain filling. Increased expression of RINO1 has been positively associated with higher phytic acid accumulation in rice grains (Perera et al., 2018). Transgenic studies involving suppression of RINO1 expression have resulted in substantial reductions in grain phytate content, confirming its central role in regulating phytic acid biosynthesis (Suzuki et al., 2007). The MIPS gene encodes myo-inositol-3-phosphate synthase, the enzyme responsible for initiating inositol biosynthesis. Because MIPS catalyzes the rate-limiting step of the pathway, it is regarded as a major regulatory point controlling phytic acid accumulation (Perera et al., 2018). Kumar et al. (2020) reported elevated MIPS expression in rice varieties exhibiting high phytic acid content. Their study suggested that enhanced MIPS activity contributes directly to increased phytate accumulation and reduced starch digestibility. The Inositol Pentakisphosphate 2-Kinase (IPK1) gene catalyze the final phosphorylation step converting $InsP_5$ to phytic acid ($InsP_6$). Mutations affecting IPK1 have been associated with low-phytate phenotypes in rice and other cereal crops (Suzuki et al., 2007). Ghosh and Roychoudhury (2020) observed increased IPK1 expression in aromatic rice genotype IET-21261, which accumulated significantly higher levels of phytic acid and total phosphorus. These findings suggest that IPK1 expression may be directly associated with grain phytate concentration. IPK2 catalyzes intermediate phosphorylation reactions within the phytic acid biosynthetic pathway. Although less extensively studied than RINO1 and IPK1, IPK2 is considered essential for efficient conversion of lower inositol phosphates into higher phosphorylated intermediates (Suzuki et al., 2007). Coordinated expression of IPK2 along with MIPS and IPK1 has been reported during grain development, indicating that phytate accumulation depends on the integrated regulation of multiple biosynthetic genes.

Phytic acid is widely recognized as an anti-nutritional factor because of its strong chelating capacity toward mineral cations. It readily binds iron, zinc, calcium, magnesium, and potassium, forming insoluble phytate complexes that are poorly absorbed by the human gastrointestinal tract (Perera et al., 2018). The inability of humans and other monogastric animals to synthesize phytase limits their capacity to hydrolyse phytate complexes efficiently (Raboy, 2000). Consequently,

populations relying heavily on cereal-based diets frequently experience deficiencies of iron, zinc, and calcium despite adequate total mineral intake. Several studies have demonstrated that mineral bioavailability is influenced more strongly by phytate concentration than by total mineral content itself. Liang et al. (2006) reported that despite considerable variation in iron and zinc concentrations among Chinese rice varieties, elevated phytic acid levels substantially reduced mineral bioavailability. The negative effects of phytic acid are particularly severe among infants, pregnant women, and lactating mothers, who have increased micronutrient requirements (Al Hasan et al., 2016; Chan et al., 2007). Consequently, reducing phytate levels while maintaining adequate mineral concentrations has become a major objective of rice biofortification programs. Interestingly, phytic acid also possesses beneficial properties. Doria et al. (2009) reported that phytic acid functions as a natural antioxidant, protecting seeds against oxidative damage during storage and germination. Therefore, breeding programs aim to achieve optimal rather than extremely low phytic acid levels. Research on low-phytate rice mutants demonstrated that reduced phytic acid content enhances mineral accumulation in the endosperm. Sakai et al. (2015) observed increased concentrations of phosphorus, sulfur, potassium, and calcium within the endosperm of low-phytate rice seeds, suggesting improved mineral availability for human consumption.

Mishra et al. (2020) examined the spatial distribution of iron within rice grains and its relationship with phytic acid accumulation and ferritin gene expression. Considerable genotypic variation was observed in iron localization among the embryo, aleurone layer, and endosperm tissues. Rice genotypes characterized by lower phytic acid concentrations exhibited greater iron accumulation and improved iron bioavailability in the edible endosperm. The study identified *Oryza rufipogon* and the cultivar Sarjoo 52 as promising donor lines for breeding programs aimed at enhancing grain iron concentration and nutritional quality. These findings further support the importance of reducing phytic acid content as a strategy for improving micronutrient bioavailability in rice. Behera et al. (2026) investigated the genetic loci associated with grain quality traits and phytic acid content in aromatic rice using candidate gene-based simple sequence repeat (SSR) markers. The study evaluated 138 aromatic rice accessions and revealed substantial genetic variation in grain phytic acid concentration among the genotypes. Association analysis identified a significant relationship between markers linked to the LPA (Low Phytic Acid) gene and grain phytic acid content, indicating that natural allelic variation plays an important role in regulating phytate accumulation in aromatic rice. The authors suggested that indigenous aromatic rice genotypes

exhibiting low phytic acid content could serve as valuable donor parents in breeding programs aimed at improving mineral bioavailability while preserving desirable grain quality attributes.

Kumar et al. (2023) examined the influence of phytic acid concentration and grain processing on mineral bioavailability in rice. A total of 67 rice genotypes representing diverse categories, including aromatic, pigmented, high-protein, and conventional rice varieties, were evaluated for grain iron, zinc, and phytic acid contents. The study demonstrated that phytic acid was a major determinant of micronutrient bioavailability, irrespective of the total mineral concentration present in the grain. Several rice genotypes with high iron and zinc contents exhibited reduced mineral bioavailability due to elevated phytate levels. Furthermore, significant variation in phytic acid accumulation was observed among the different rice groups, highlighting the importance of phytate concentration in determining grain nutritional quality. The authors emphasized that the development and selection of rice varieties combining high micronutrient content with low phytic acid levels are essential for enhancing mineral absorption and improving the nutritional value of rice-based diets.

Emami et al. (2010) compared the protein expression profiles of a low-phytic acid rice mutant with its corresponding parental line using proteomic and bioinformatic approaches. The study identified several differentially expressed proteins associated with stress responses, energy metabolism, storage protein synthesis, and cellular regulatory processes. Proteomic analysis revealed altered expression of enzymes such as fructose-bisphosphate aldolase and triose phosphate isomerase, which participate in carbohydrate metabolism and are indirectly linked to myo-inositol biosynthesis and phytic acid accumulation. In addition, a significant reduction in globulin storage protein accumulation was observed in the low-phytate genotype. The authors concluded that integrated proteomic and bioinformatic analyses provide valuable tools for functional genomics studies and contribute to a better understanding of the molecular mechanisms underlying low-phytic acid phenotypes in rice.

Liang et al. (2006) evaluated the variation in phytic acid, iron, and zinc contents among 56 rice varieties collected from major rice-growing regions of China and assessed their implications for mineral bioavailability. Considerable variability was observed among the genotypes, with iron concentrations ranging from 9 to 45 mg kg^{-1}, zinc concentrations from 13 to 39 mg kg^{-1}, and phytic acid contents from 7.2 to 11.9 g kg^{-1}. The study found no significant association between iron

concentration and phytic acid content or grain morphology. However, zinc concentration showed a positive correlation with phytic acid levels and was influenced by both grain characteristics and geographical origin. The molar ratios of phytic acid to iron and phytic acid to zinc ranged from 15–105 and 27–67, respectively, indicating poor mineral bioavailability. Based on these ratios, the authors estimated that the bioavailability of iron and zinc in the studied rice varieties was generally below 4%, largely due to the inhibitory effects of phytic acid on mineral absorption. The findings showed that although genetic and regional differences contribute to variation in mineral concentrations, elevated phytate levels remain a major constraint to micronutrient utilization. Therefore, it's recommended to combine varietal selection, agronomic management, and post-harvest processing strategies to reduce phytic acid levels while retaining essential mineral nutrients, thereby enhancing the nutritional quality of rice. These studies collectively demonstrate that phytic acid plays a central role in determining mineral bioavailability and nutritional quality in rice.

Variations in the expression of phytic acid biosynthetic genes may influence phytic acid accumulation among different rice genotypes. Aromatic and non-aromatic rice varieties possess distinct genetic backgrounds and metabolic characteristics, which may affect the regulation of phytic acid biosynthesis. Hence, the present study was formulated to investigate the expression patterns of key phytic acid biosynthetic genes, namely OsPGK1, RINO1, OsMIK, OsITPK1, and OsMRP13, in selected aromatic and non-aromatic rice varieties using quantitative real-time PCR (qRT-PCR). Understanding the differential expression of these genes will provide insights into the molecular mechanisms governing phytic acid accumulation and may contribute to the development of nutritionally improved rice cultivars with enhanced mineral bioavailability.

Chapter 3
Aim and Objectives

Aim

To evaluate the expression patterns of phytic acid biosynthetic genes in selected aromatic and non-aromatic rice varieties using quantitative real-time PCR.

Objectives

- To isolate RNA and synthesize cDNA from aromatic and non-aromatic rice varieties
- To quantify the expression of OsPGK1, RINO1, OsMIK, OsITPK1, and OsMRP13 genes using qRT-PCR.
- To compare gene expression levels between aromatic and non-aromatic rice varieties.
- To assess the relationship between gene expression patterns and phytic acid biosynthesis in selected rice.

Chapter 4
Materials and Methods

4.1 Plant Materials

Four rice (*Oryza sativa* L.) varieties representing aromatic and non-aromatic groups were used in this study (Table 1).

Table 1: Details of rice varieties selected for the study

Sl. No.	Rice Variety	Class	Collection Site
1	Jeerakasala	Aromatic	Farmers' plot, Wayanad
2	Basmati	Aromatic	Farmers' plot, Wayanad
3	Uma	Non-aromatic	Rice Research Station, KAU, Moncompu, Alappuzha
4	Pratyasa	Non-aromatic	Rice Research Station, KAU, Moncompu, Alappuzha

The aromatic varieties included Jeerakasala and Basmati, while the non-aromatic varieties comprised Pratyasa, and Uma. Morphology of the selected rice varieties were shown in Figure 2. Variations in grain size, shape, colour, and overall appearance were observed among the varieties, reflecting their distinct genetic backgrounds and agronomic characteristics. These morphological differences contribute to varietal diversity and may influence grain quality attributes such as cooking behaviour, texture, and nutritional properties.

4.1.1 Sample preparation for total RNA isolation from leaves

For RNA isolation, rice grains of the selected varieties were first subjected to germination under controlled conditions. Healthy and uniform grains were washed thoroughly with distilled water and placed in a clean, moist cotton cloth. The grains were wrapped securely and incubated in darkness at room temperature for three days to facilitate uniform sprouting. To prevent microbial contamination and maintain adequate moisture, the grains were washed with sterile distilled water on alternate days during the germination period.



Figure 2: Grain morphology of the rice varieties used in the study. Representative images of varieties showing variations in grain size, shape, colour, and overall appearance, indicating their distinct genetic and phenotypic characteristics



Figure 3: Twelve-day-old seedlings of the selected rice varieties used for the present study

After three days, the sprouted grains were transferred to pots containing sterilized paddy soil and maintained under suitable growth conditions. The seedlings were watered regularly to ensure

healthy growth. On the twelfth day after sowing, young, actively growing leaves were collected for RNA extraction (Figure 3).

4.2 Reagents, Consumables and Equipment used for the study

4.2.1 Reagents for RNA Isolation

TRIzol reagent (Invitrogen, USA), chloroform, isopropanol, 95% ethanol, diethyl pyrocarbonate (DEPC)-treated water, RNase-Zap, and nuclease-free water were used for RNA isolation. All reagents were of molecular biology grade and prepared according to the manufacturer's recommendations.

4.2.2 Consumables and Equipment

The consumables and equipment used for RNA isolation included sterile RNase-free microcentrifuge tubes, aerosol-resistant pipette tips, mortar and pestle, and liquid nitrogen for tissue grinding. All glassware and working surfaces were treated to minimize RNase contamination.

4.2.3 Reagents for RNA Quality Assessment

Agarose, 1X-Tris-acetate-EDTA (TAE) buffer, Ethidium bromide (EtBr), and 6× gel loading dye containing 40% sucrose, Xylene Cyanol and Bromophenol blue.

4.2.4 Requirements for Gradient PCR

Emerald Green PCR Master mix (Takara, USA), 100 bp DNA ladder (NEB, USA), Agarose gel (same as 4.2.3).

4.2.5 Reagents for First-Strand cDNA Synthesis

First-strand cDNA synthesis was performed using the iScript™ cDNA Synthesis Kit (Bio-Rad, USA), containing iScript™ RT Super mix with reverse transcriptase, reaction buffer, and oligo(dT) primers.

4.3 Plant Material Development and Sample Preparation for RNA Isolation

Seeds of the selected rice varieties were initially cleaned and soaked in distilled water for 24 hours. The soaked seeds were then wrapped in a moist cotton cloth and maintained under ambient

conditions to facilitate germination. The seeds were rinsed with distilled water twice daily to maintain adequate moisture. After three days, uniformly sprouted seeds were selected and sown in pots containing sterilized paddy soil.

The seedlings were grown under controlled environmental conditions in a growth chamber maintained at 28 ± 2°C with a 16 h light/8 h dark photoperiod and relative humidity of 60–70%. Plants were watered regularly to maintain optimum soil moisture throughout the growth period. Twelve-day-old healthy seedlings exhibiting uniform growth were selected for gene expression analysis. Young leaf tissues were harvested on the twelfth day from the selected seedlings during the same time period of the day to minimize variations due to circadian regulation of gene expression. The collected leaf samples were immediately frozen in liquid nitrogen and used for RNA isolation.

4.4 Isolation of Total RNA Using TRIzol Method

Total RNA was extracted from 500 mg of rice leaf tissue using TRIzol reagent (Invitrogen, USA) following the manufacturer's protocol with slight modifications optimized for rice tissues.

- Total RNA was extracted from 500 mg of leaf tissue using the TRIzol reagent following optimized modifications for rice tissues. All centrifugation steps were performed at $4^{O}C$.
- Disrupt samples to a fine powder in liquid nitrogen using a chilled mortar and pestle.
- Homogenize the powder in 1 mL of TRIzol reagent.
- Vortex the homogenate and incubate at room temperature for 5 min to allow complete dissociation of nucleoprotein complexes.
- Add 200 µL chloroform per 1 mL of TRIzol. Shake vigorously by hand for 15 s and incubate at room temperature for 3 min.
- Centrifuge at 12,000 rpm (12,000 x g) for 15 min at $4^{O}C$ for phase separation.
- Carefully transfer the upper aqueous phase containing the RNA to fresh 1.5 mL tubes without disturbing the interphase.
- Precipitate the RNA by mixing gently with 500 µL (0.5 mL) of isopropanol per 1 mL of TRIzol.
- Incubate the mixture at room temperature for 10 min.
- Centrifuge at 12,000 rpm (12,000 x g) for 10 min at $4^{O}C$.

- Remove the supernatant and wash the RNA pellets with 1 mL of 75% ethanol.
- Vortex once and centrifuge at 2000 rpm for 4 min at 4^{O}C.
- Discard the supernatant and allow the pellet to dry for 5 min at room temperature.
- Dissolve the RNA pellets in 50 µL of DEPC-treated water, transfer into 0.5 mL tubes in 10 µL aliquots, and store at -80^{O}C.

4.5 Determination of RNA Quality

RNA concentration and purity were determined using a Bio-Photometer (Eppendorf, Germany). Purity was assessed by measuring absorbance ratios at A260/A280 and A260/A230. It measures nucleic acid concentration and purity based on the absorbance of ultraviolet (UV) light by RNA molecules. RNA absorbs UV light maximally at 260 nm due to the presence of aromatic nitrogenous bases. According to the Beer–Lambert law, absorbance is directly proportional to the concentration of nucleic acids in the sample.

The purity of RNA is assessed by measuring absorbance at 260 nm, 280 nm, and 230 nm:

- A260: Measures nucleic acid concentration.
- A280: Indicates protein contamination (mainly aromatic amino acids).
- A230: Indicates contamination by phenol, carbohydrates, polysaccharides, guanidine salts, or other organic compounds.
- A260/A280 ratio should be between 1.8 and 2.1.
- A260/A230 ratio should be approximately 2.0–2.2.

The concentration of RNA is calculated using the conversion factor:

$$\text{RNA concentration (µg/mL)} = A_{260} \times 40 \times \text{Dilution Factor}$$

4.6 Integrity assessment of RNA using Agarose Gel Electrophoresis

The integrity of total RNA isolated, DNA contamination and the extend of degradation were assessed through 1.2% agarose gel. It was prepared by dissolving 1.2 g agarose in 100 mL of 1× TAE buffer. The solution was heated until complete dissolution and allowed to cool before adding ethidium bromide (intercalating dye). The molten agarose was poured into a gel casting tray and allowed to solidify. RNA samples mixed with 6X- gel loading dye were loaded into the wells and electrophoresed at 70 V for about 40 minutes when the separation was achieved. The gel was

visualized using a GelDoc GO Imaging System (Bio-Rad, USA), and images were documented for quality assessment.

4.7 First-Strand cDNA Synthesis

First-strand complementary DNA (cDNA) was synthesized from 1 µg of total RNA using the iScript™ cDNA Synthesis Kit (Bio-Rad Laboratories, USA) according to the manufacturer's instructions.

cDNA Synthesis Reaction Mixture

Component	Volume (µL)
5× iScript Reaction Mix	4
iScript Reverse Transcriptase	1
RNA Template (1 µg)	Variable
Nuclease-free Water	Up to 20

Thermal Cycling Conditions for cDNA Synthesis

Step	Temperature	Time
Primer Annealing	25°C	5 min
Reverse Transcription	46°C	20 min
Enzyme Inactivation	95°C	1 min

4.8 Selection of Candidate Genes and Primer Design

Genes involved in phytic acid biosynthesis, phosphorylation, and transport pathways were selected for expression analysis. List of primers were given in Table 2.

Table 2: Primers used for quantitative real-time PCR

Gene	Forward Primer (5′–3′)	Reverse Primer (5′–3′)	Concentration used for 1 reaction
Actin (Reference Gene)	GAACAACTGGGACGACAT	CCTTTGGGTTCAGAGGAG	10 picomoles/µl
OsPGK1	CGCTTCTTGCCTTGCTTTGA	GCCTTGCATCCCATGAGTTG	10 picomoles/µl
RINO1	CCGTGGCATGTGGCAAAGAG	TGCATAGCCCGATAAGAGTC	10 picomoles/µl
OsMIK	TCCATCCATGGCGCCCTCTC	TCCACCTCAACCACCACCACTTC	10 picomoles/µl
OsITPK1	TCCTACTCAGCCTTGTGC	TGACTGCTAGACCGTTAC	10 picomoles/µl
OsMRP13	GGAGCACTACCAAGATAAGC	ATTGGTGAACTCCAGAAAAC	10 picomoles/µl

Gene-specific primers were obtained from published literature (Desingu et al., 2025) and synthesized commercially. Rice β-actin (OsAct) was used as the endogenous reference gene for normalization. Working primer solutions (10 pmol/µL) were prepared from 100 pmol/µL stock solutions by a 1:10 dilution using nuclease-free water. For each PCR reaction, 10 pmol/µL of both forward and reverse primers were used. All primers were standardised for annealing temperature using gradient PCR (Temperature range 55ºC to 62ºC).

4.9 Quantitative Real-Time PCR (qRT-PCR)

Quantitative real-time PCR was performed using a Bio-Rad CFX96 Real-Time PCR Detection System (Bio-Rad, USA) with SYBR Green chemistry. Each reaction was carried out in a final volume of 20 µL containing SYBR Green Master Mix, gene-specific primers, cDNA template, and nuclease-free water.

All reactions were performed in triplicate.

qRT-PCR Thermal Cycling Conditions

Step	Temperature	Time
Initial Denaturation	95°C	3 min
Denaturation (40 cycles)	95°C	15 s
Annealing	60°C	30 s
Extension	72°C	30 s

A melt curve analysis was performed after amplification to verify product specificity and the absence of primer-dimer formation (Bustin et al., 2009).

4.10 Analysis of Relative Gene Expression

Relative gene expression was determined using the comparative Ct (2^−ΔΔCt) method described by Livak and Schmittgen (2001). This method enables the determination of changes in gene expression levels between experimental samples and a designated control sample after normalization against a stable internal reference gene. In the present study, β-Actin was used as the endogenous reference gene because of its constitutive and stable expression across different rice genotypes and experimental conditions. The reference gene served to normalize variations arising from differences in RNA quantity, RNA quality, and cDNA synthesis efficiency among samples. Quantitative real-time PCR reactions were carried out in triplicate for each biological

sample. The cycle threshold (**Ct**) values obtained from the qRT-PCR instrument were recorded, and the mean Ct value of the three technical replicates was used for expression profiling analysis.

The relative expression of the gene selected was calculated using the following steps:

Step 1: Calculation of ΔCt

For each sample, the Ct value of the reference gene (β-Actin) was subtracted from the Ct value of the target gene (phytic acid biosynthesis gene).

$$\Delta Ct = Ct_{(target\ gene)} - Ct_{(\beta\text{-}Actin)}$$

This normalization step corrects for sample-to-sample variations in template concentration.

Step 2: Calculation of ΔΔCt

The ΔCt value of each test sample was compared with the ΔCt value of the control sample to determine the relative change in expression:

$$\Delta\Delta Ct = \Delta Ct_{(Test)} - \Delta Ct_{(Control)}$$

The control sample served as the calibrator (Uma) against which all other samples were evaluated.

Step 3: Calculation of Relative Gene Expression

The fold change in candidate genes involved in phytic acid biosynthetic gene expression relative to the control was determined using the following equation:

$$\text{Relative Expression (Fold Change)} = 2^{-\Delta\Delta Ct}$$

A fold-change value greater than 1 indicated upregulation of gene expression relative to the control, whereas a value less than 1 indicated downregulation. A fold-change value equal to 1 represented no significant change in expression. The calculated relative expression values were used to compare phytic acid biosynthesis related transcript abundance among the selected rice varieties. The results were expressed as mean fold-change values derived from three technical replicates and were subsequently used for statistical analysis and graphical representation.

4.11 Statistical Analysis

All qRT-PCR reactions were performed in triplicate. Mean Ct values were used for ΔCt, ΔΔCt, and fold-change calculations. Data were expressed as mean ± standard error (SE). Statistical analyses and graphical representations were performed using Microsoft Excel.

Chapter 5

Results

5.1 Isolation of Total RNA from Rice Leaf Tissues using TRIzol method

The standard TRIzol reagent method yielded high quality total RNA from the metabolically active leaf tissues of the four selected rice varieties (Jeerakasala, Basmati, Uma, Pratyasa). This method proved to be effective in breaking down the rigid plant cell walls and inhibiting the endogenous ribonucleases thus enabling the successful partitioning and recovery of pure RNA from the complex cellular matrix.

5.2 RNA Quality Assessment

The quality of the isolated RNA was evaluated prior to cDNA synthesis to ensure its suitability for downstream gene expression studies.

5.2.1 Quantification and Quality assessment

The isolated total RNA was quantified and quality was evaluated for concentration and biochemical purity using a biophotometer.

Table 3: Quantitative estimation of total RNA extracted from traditional and released rice leaf tissues.

Rice Variety	Classification	Concentration (ng/µL)	A260/A280 Ratio	A260/A230 Ratio
Jeerakasala	Aromatic	685.4	2.02	2.15
Basmati	Aromatic	712.8	1.98	2.08
Uma	Released	650.2	2.05	2.11
Pratyasa	Released	745.6	2.01	2.18

Absorbance readings were taken at 260 nm (for nucleic acids), 280 nm (for proteins) and 230 nm (for polyphenols and polysaccharides). The RNA yields were sufficient for downstream

applications. The purity of RNA was confirmed by A260/280 absorbance ratios between 1.98 and 2.05 (Table 3), indicating minimal protein contamination. All rice varieties used for the study yielded with high RNA concentrations (650.2–745.6 ng/µL) and efficient recovery from complex tissues. Ratios of 1.98-2.05 confirmed purity and removal of protein contaminants, while ratios of 2.08-2.18 confirmed elimination of plant-specific inhibitors. These metrics confirm RNA integrity and suitability for sensitive downstream applications like qRT-PCR.

5.3 Integrity Assessment of RNA using Agarose Gel Electrophoresis

While bio photometric analysis confirms purity and concentration, it cannot distinguish between intact and degraded RNA. Therefore, Agarose Gel Electrophoresis (AGE) was performed using a 1.2% denaturing agarose gel to visually assess the physical integrity of the extracted total RNA. The RNA samples showed distinct 28S and 18S rRNA bands on 1.2% agarose gel electrophoresis, indicating good integrity (Figure 4).

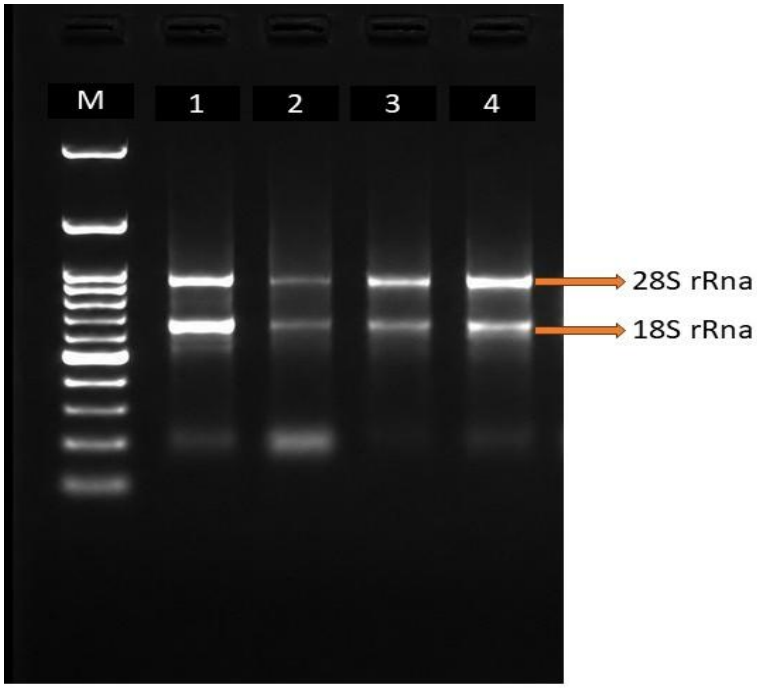


Figure 4: Agarose gel electrophoresis (1.2%) demonstrating the structural integrity of total RNA isolated from the leaf tissues of (Lane 2) Jeerakasala, (Lane 3) Basmati, (Lane 4) Uma, and (Lane 5) Pratyasa

5.4 cDNA Quality Assessment Using β-Actin Reference Gene

The quality and integrity of the synthesized cDNA were evaluated by quantitative real-time PCR (qRT-PCR) using the β-actin reference gene. The amplification profile exhibited a characteristic sigmoid-shaped curve with a Ct value of 24.3, indicating successful cDNA synthesis and adequate template quantity for downstream gene expression analysis. Melt curve analysis revealed a single, sharp melting peak, confirming the amplification of a specific PCR product without any non-specific amplification or primer-dimer formation. These results demonstrate that the synthesized cDNA was of high quality and suitable for reliable and reproducible quantification of target gene expression in the selected rice varieties (Figure 5).

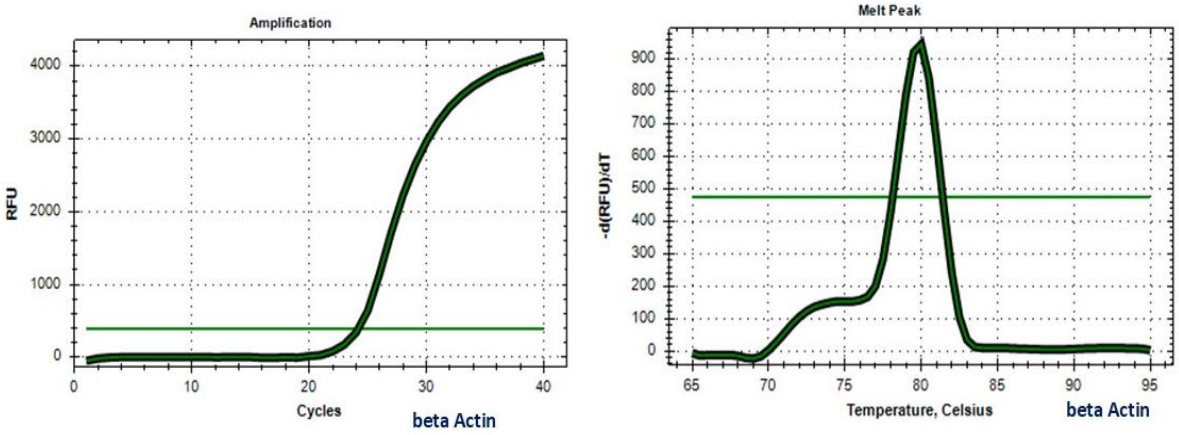


Figure 5: Representative qRT-PCR amplification and melt curve analysis of the β-actin reference gene used for cDNA quality assessment.

The reliability of quantitative real-time PCR (qRT-PCR) data is fundamentally dependent on the stability of the internal reference (housekeeping) gene used for normalization. In this study, the β -actin (ACT) gene was tested across all biological replicates and experimental runs.

5.5 Gene Expression Profiling

To elucidate the molecular basis of phytic acid biosynthesis among the selected rice varieties, the relative expression levels of five key genes involved in the phytic acid biosynthetic pathway OsMIK, OsITPK, OsPGK1, RINO1, and OsMRP13were analysed using quantitative real-time PCR (qRT-PCR). The analysis was performed using gene-specific primers, with relative transcript abundance determined after normalization against the reference gene Actin. All gene-specific

primers exhibited efficient and specific amplification, with no evidence of non-specific products or primer-dimer formation.

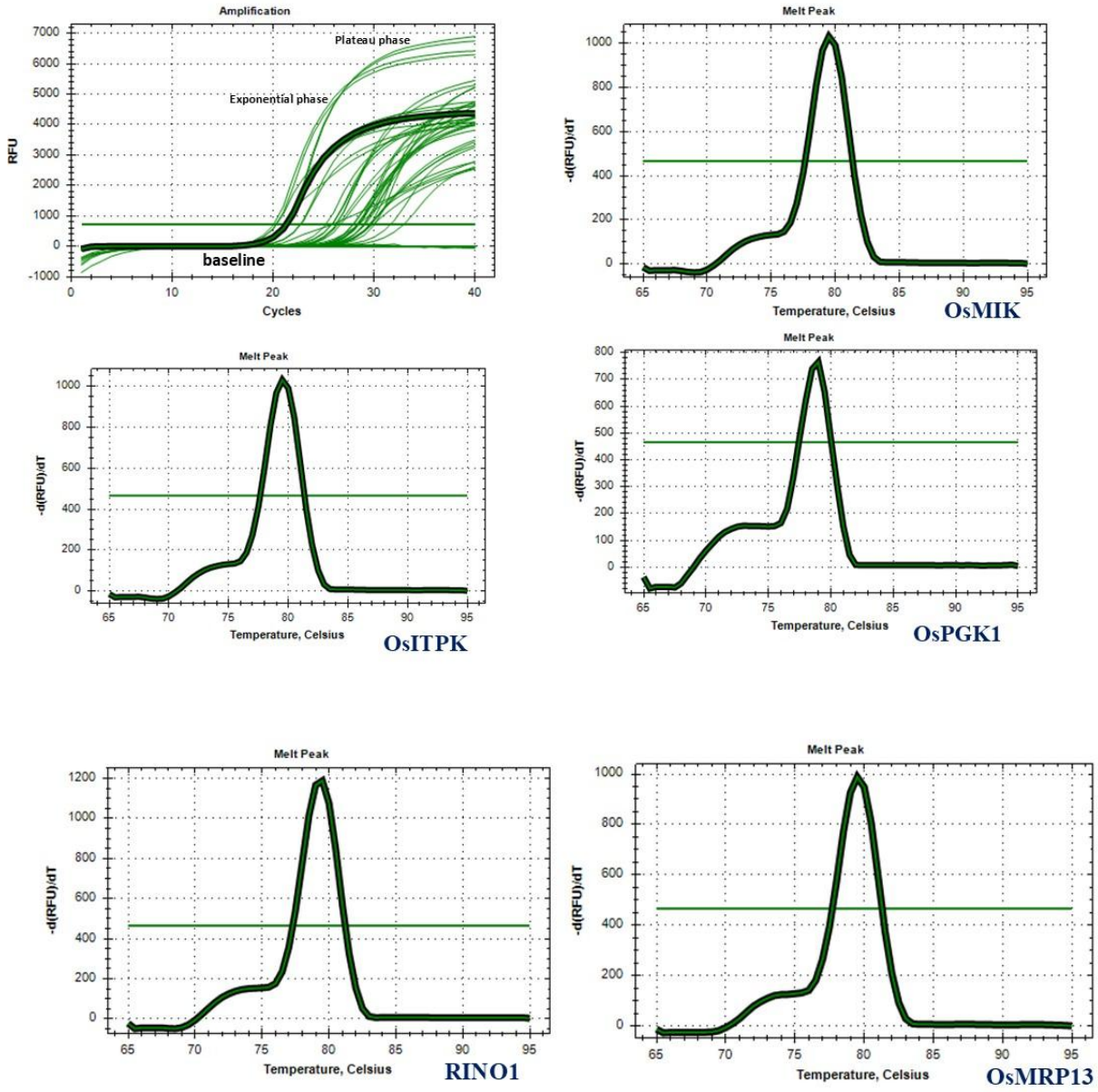


Figure 6: Representative amplification plot showing the typical sigmoid amplification curves and melt curve data for the primers used for the study

Representative amplification plots showed characteristic sigmoid-shaped curves, indicating efficient exponential amplification and consistent fluorescence signal accumulation throughout the PCR cycles. The absence of aberrant amplification patterns confirmed the specificity and reliability of the primers, ensuring accurate quantification of gene expression. A representative

amplification plot illustrating the typical sigmoid amplification curves is presented in Figure 6. Subsequent analysis revealed distinct genotype-dependent differences in the expression patterns of the five target genes, reflecting variation in the regulation of phytic acid biosynthesis among the rice varieties (Figure 6).

5.5.1 Relative Expression Analysis of the Phytic Acid Pathway Gene *OsPGK1*

Quantitative real-time PCR (qRT-PCR) analysis revealed significant variation in the relative expression of the OsPGK1 gene among the selected rice varieties (Figure 7). The released variety Uma, which served as the control, was assigned a normalized expression value of 1.0 (2^-ΔΔCt).

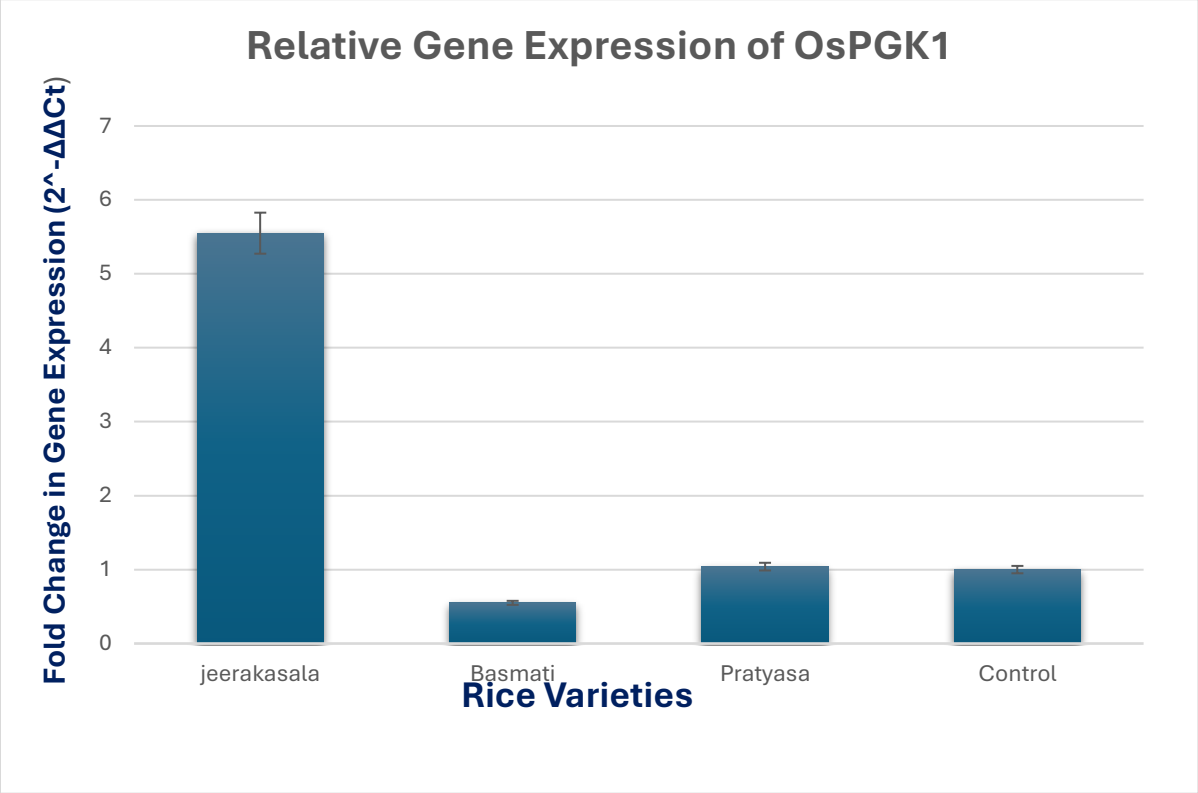


Figure 7: Relative expression analysis of the OSPGK1 gene in selected rice varieties determined by quantitative real-time PCR (qRT-PCR). Relative transcript levels were calculated using the 2^-ΔΔCt method with Actin as the internal reference gene and Uma as the calibrator (control; fold change = 1)

Among the test genotypes, the traditional aromatic rice Jeerakasala exhibited the highest level of OsPGK1 expression, showing an approximately 5.5-fold increase relative to the control. This marked upregulation indicates enhanced transcriptional activity of OsPGK1, suggesting a greater metabolic flux through the phytic acid biosynthetic pathway in Jeerakasala. In contrast, the aromatic rice Basmati exhibited the lowest expression level, with a relative fold change of

approximately 0.5, representing nearly a 50% reduction compared to the control. This downregulation suggests comparatively lower expression of OsPGK1 and may indicate reduced contribution of this gene to phytic acid biosynthesis in Basmati.

The released variety Pratyasa displayed an expression level comparable to the control (approximately 1.0-fold), indicating that OsPGK1 transcript abundance remained relatively unchanged between these two improved cultivars. The relatively larger error bars observed for Pratyasa and the control indicate greater biological variability among replicates, whereas Jeerakasala and Basmati exhibited more consistent expression patterns. The results demonstrate clear genotype-dependent differences in OsPGK1 expression. The pronounced upregulation observed in the aromatic traditional variety Jeerakasala, compared with both the aromatic Basmati and the released varieties (Uma and Pratyasa), suggests that regulation of OsPGK1 differs substantially among rice genotypes. Since OsPGK1 encodes phosphoglycerate kinase, an enzyme involved in glycolysis that provides metabolic intermediates and ATP required for the synthesis of myo-inositol, the precursor for phytic acid biosynthesis, its elevated expression in Jeerakasala may contribute to enhanced phytic acid accumulation. Conversely, the reduced expression in Basmati may reflect lower metabolic commitment toward phytic acid synthesis. These findings indicate that both aromatic and non-aromatic rice varieties possess distinct transcriptional regulation of OsPGK1, which may influence differences in phytic acid content and phosphorus metabolism among the selected genotypes.

5.5.2 Relative Expression Analysis of the Phytic Acid Pathway Gene *RINO1*

Quantitative real-time PCR (qRT-PCR) analysis demonstrated considerable variation in the expression of the RINO1 gene among the selected rice varieties (Figure 8). The released variety Uma, used as the control, was assigned a normalized relative expression value of 1.0 (2^-ΔΔCt). Among the evaluated genotypes, the traditional aromatic rice Basmati exhibited the highest RINO1 transcript abundance, with an approximately 1.8-fold increase relative to the control, indicating significant upregulation of this key gene involved in the initial steps of phytic acid biosynthesis.

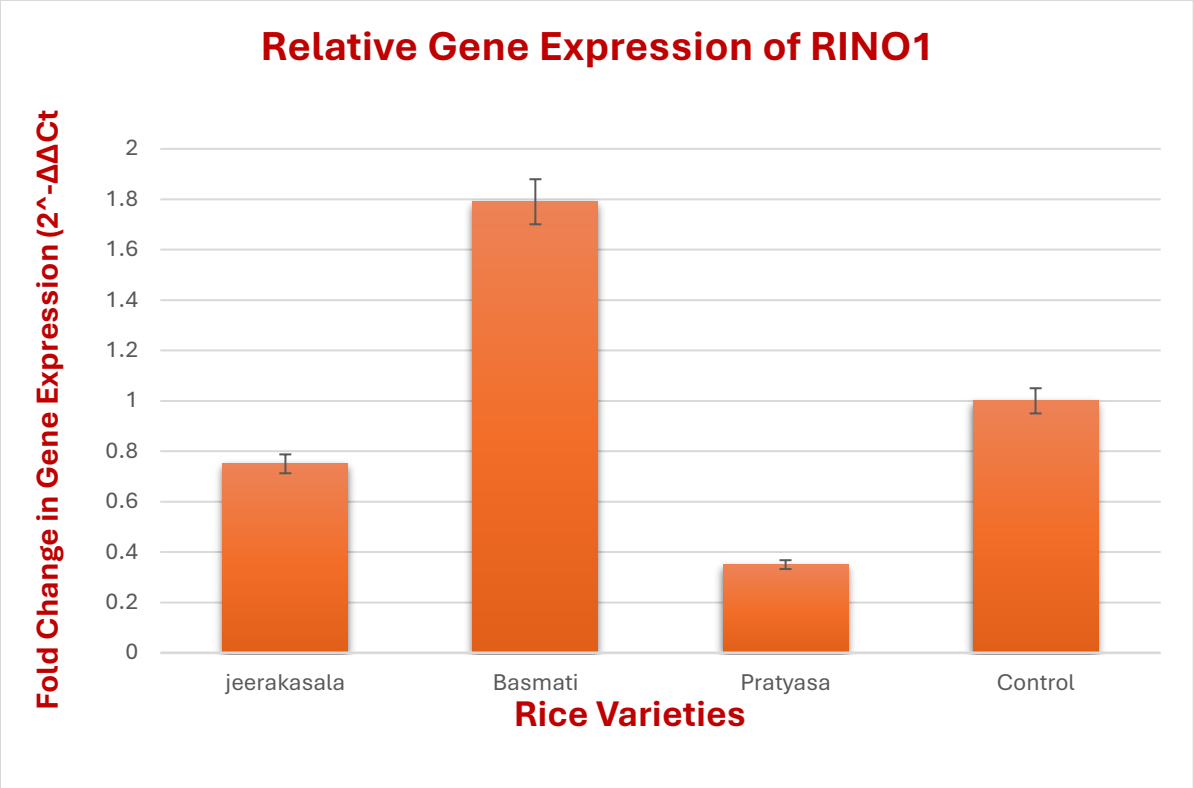


Figure 8: Relative expression analysis of the RINO1 gene in selected rice varieties determined by quantitative real-time PCR (qRT-PCR). Relative transcript levels were calculated using the 2^-ΔΔCt method with Actin as the internal reference gene and Uma as the calibrator (control; fold change = 1)

In contrast, the traditional aromatic rice Jeerakasala showed a moderate reduction in RINO1 expression, with a relative fold change of approximately 0.75, corresponding to nearly 25% lower expression than the control. The released variety Pratyasa exhibited the lowest expression level, with a relative fold change of approximately 0.35, indicating substantial downregulation compared with Uma. The larger error bars observed for Basmati, Pratyasa, and the control suggest greater biological variation among replicates, whereas Jeerakasala displayed comparatively consistent expression.

The observed expression pattern indicates that RINO1 is differentially regulated among aromatic and non-aromatic rice varieties. RINO1 encodes myo-inositol-3-phosphate synthase (MIPS), the enzyme responsible for catalysing the conversion of glucose-6-phosphate to myo-inositol-3-phosphate, the first committed and rate-limiting step in the phytic acid biosynthetic pathway. The elevated expression of RINO1 in Basmati suggests enhanced biosynthesis of myo-inositol and a potentially greater metabolic capacity for phytic acid production. Conversely, the reduced expression observed in Jeerakasala and particularly in Pratyasa indicates comparatively lower

transcriptional activity of this key regulatory gene. Results of the study revealed that distinct genotype-dependent regulation of RINO1 among the selected rice varieties. Interestingly, although both Jeerakasala and Basmati are aromatic rice cultivars, they exhibited contrasting expression profiles, with Basmati showing marked upregulation and Jeerakasala showing reduced expression relative to the control. Similarly, the two released varieties also differed in their expression patterns, with Uma maintaining the baseline expression and Pratyasa exhibiting significant downregulation. These findings suggest that the transcriptional regulation of RINO1 is genotype-specific rather than being determined solely by aromatic or non-aromatic classification, and may contribute to varietal differences in phytic acid accumulation and phosphorus metabolism.

5.5.3 Relative Expression Analysis of the Phytic Acid Pathway Gene *OsMIK*

Quantitative real-time PCR (qRT-PCR) analysis revealed substantial variation in the expression of the OsMIK gene among the selected rice varieties (Figure 9). The released variety Uma, used as the calibrator (control), was assigned a normalized relative expression value of 1.0 (2^-ΔΔCt). Among all the tested genotypes, the traditional aromatic rice Jeerakasala exhibited the highest OsMIK expression, with an approximately 7.0-fold increase relative to the control, indicating strong upregulation of this gene. Basmati, another aromatic rice variety, also showed elevated expression, with an approximately 2.8-fold increase, although considerably lower than that observed in Jeerakasala. The released variety Pratyasa displayed a moderate increase in expression, with a relative fold change of approximately 2.4, indicating higher transcript abundance than the control but lower than both aromatic varieties.

The expression profile demonstrates that OsMIK is positively regulated in all the evaluated rice varieties compared with the control, with the degree of upregulation varying considerably among genotypes. The relatively larger error bars observed for Basmati and Pratyasa indicate greater biological variability among replicates, whereas Jeerakasala exhibited consistently high expression levels.

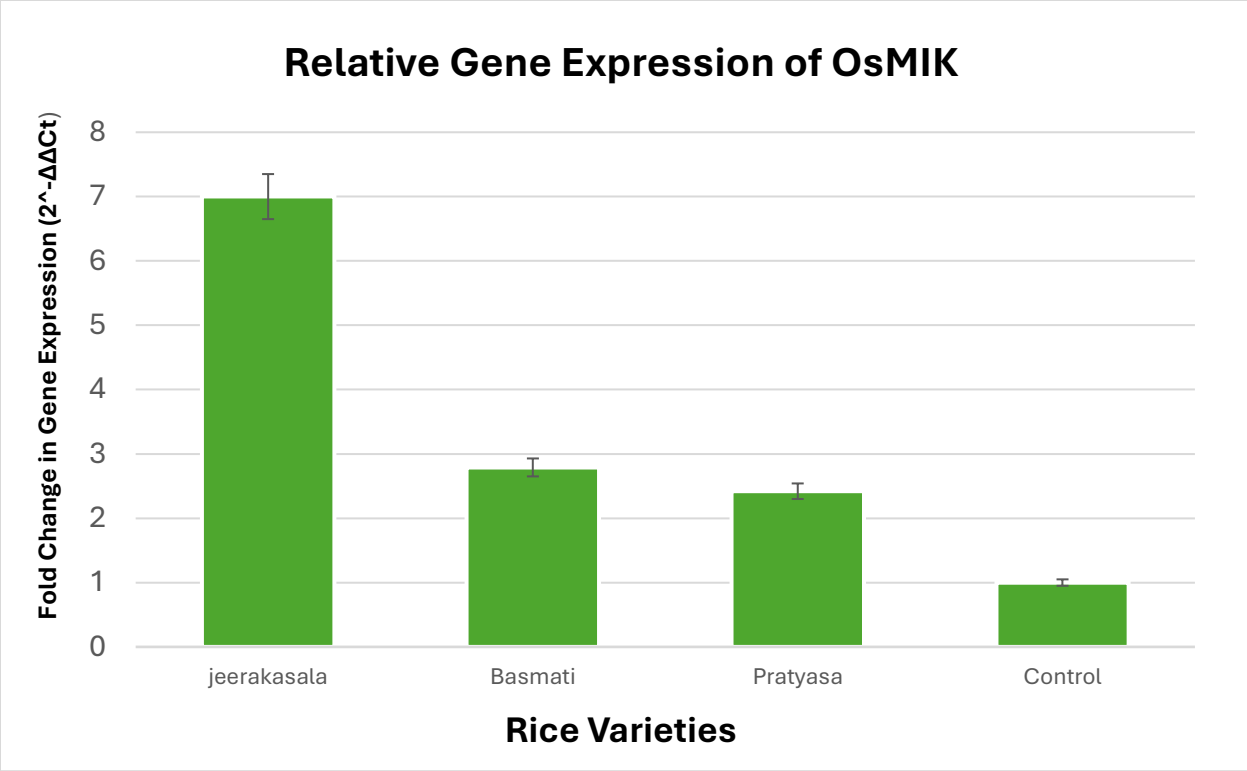


Figure 9: Relative expression analysis of the OsMIK gene in selected rice varieties determined by quantitative real-time PCR (qRT-PCR). Relative transcript levels were calculated using the 2^-ΔΔCt method with GAPDH as the internal reference gene and Uma as the calibrator (control; fold change = 1)

OsMIK encodes myo-inositol kinase, which catalyzes the phosphorylation of myo-inositol to produce inositol monophosphate, an important intermediate in the phytic acid biosynthetic pathway. The pronounced upregulation of OsMIK in Jeerakasala suggests enhanced metabolic activity toward phytic acid biosynthesis and inositol phosphate metabolism. The moderate increase observed in Basmati indicates that although both aromatic rice varieties possess elevated OsMIK expression, the magnitude of induction differs substantially between them. Similarly, the released variety Pratyasa exhibited higher expression than the control, suggesting activation of the pathway, although to a lesser extent than the aromatic genotypes. The representations indicate clear genotype-dependent regulation of OsMIK among the selected rice varieties. The exceptionally high expression in the traditional aromatic variety Jeerakasala, followed by Basmati and Pratyasa, suggests that transcriptional regulation of OsMIK differs among aromatic and released rice cultivars. These differences may contribute to variation in phytic acid biosynthesis, phosphorus storage, and seed nutritional quality, highlighting OsMIK as an important regulatory gene influencing phytic acid metabolism in rice.

5.5.4 Relative Expression Analysis of the Phytic Acid Pathway Gene *OsITPK1*

Quantitative real-time PCR (qRT-PCR) analysis revealed differential expression of the OsITPK1 gene among the selected rice varieties (Figure 10). The released variety Uma, used as the calibrator (control), was assigned a normalized relative expression value of 1.0 (2^-ΔΔCt). Among the evaluated genotypes, the traditional aromatic rice Jeerakasala exhibited the highest OsITPK1 transcript abundance, with an approximately 4.0-fold increase relative to the control. The aromatic variety Basmati also showed marked upregulation, with an expression level of approximately 3.6-fold, although slightly lower than that of Jeerakasala. The released variety Pratyasa displayed moderate upregulation, with an approximately 1.7-fold increase compared with the control.

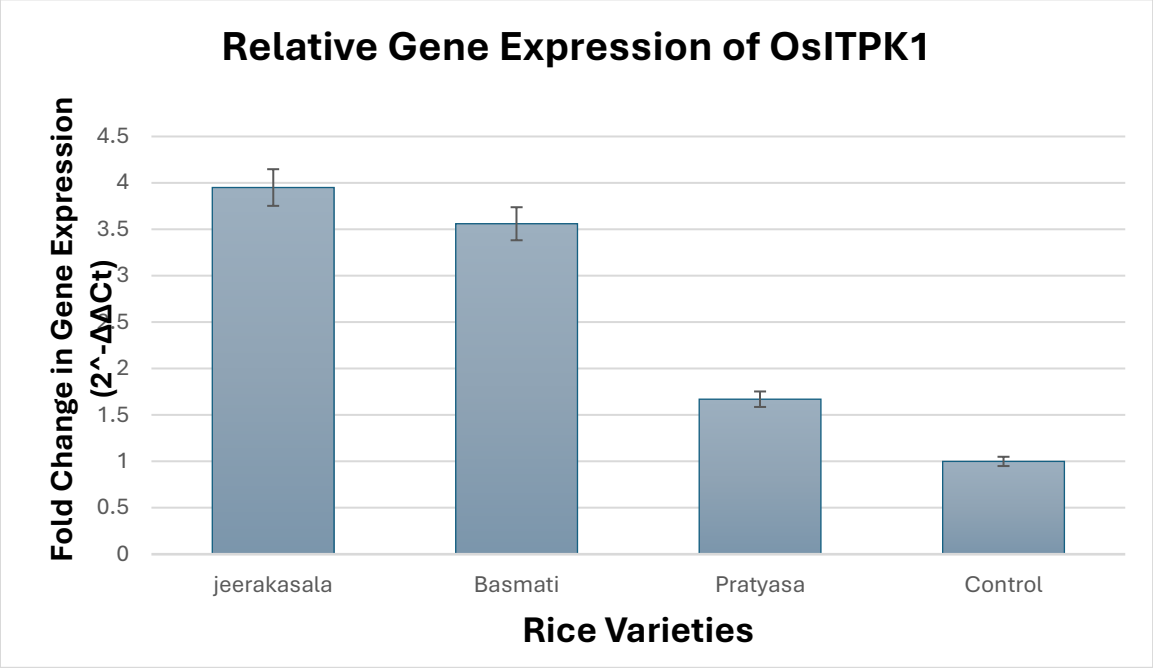


Figure 10: Relative expression analysis of the OsITPK1 gene in selected rice varieties determined by quantitative real-time PCR (qRT-PCR). Relative transcript levels were calculated using the 2^-ΔΔCt method with actin as the internal reference gene and Uma as the calibrator (control; fold change = 1)

The expression profile indicates that OsITPK1 is upregulated in all the tested rice varieties relative to the control, with the highest expression observed in the traditional aromatic cultivars. The error bars indicate moderate biological variation among replicates, particularly in Jeerakasala, Basmati, and Pratyasa, while the control exhibited relatively consistent expression.

OsITPK1 encodes inositol 1,3,4-trisphosphate 5/6-kinase, a key enzyme involved in the sequential phosphorylation of inositol phosphates during the later stages of the phytic acid biosynthetic

pathway. The significantly higher expression of OsITPK1 in Jeerakasala and Basmati suggests enhanced conversion of intermediate inositol phosphates toward higher phosphorylated forms, ultimately contributing to increased phytic acid synthesis. In contrast, the comparatively lower expression observed in Pratyasa indicates reduced transcriptional activity of this gene, although its expression remained above the control level. Comparison of aromatic and released rice varieties revealed distinct genotype-dependent regulation of OsITPK1. Both aromatic cultivars (Jeerakasala and Basmati) exhibited substantially higher expression than the released varieties (Uma and Pratyasa), suggesting that aromatic rice possesses a greater transcriptional activation of this important phytic acid pathway gene. These findings indicate that variation in OsITPK1 expression may contribute to differences in phytic acid accumulation, phosphorus storage, and mineral bioavailability among rice genotypes, highlighting its potential role as an important molecular marker for improving grain nutritional quality.

5.5.5 Relative Expression Analysis of the Phytic Acid Pathway Gene *OsMRP13*

Quantitative real-time PCR (qRT-PCR) analysis revealed genotype-dependent differences in the expression of the OsMRP13 gene among the selected rice varieties (Figure 11). The released variety Uma, used as the calibrator (control), was assigned a normalized relative expression value of 1.0 (2^-ΔΔCt). Among the evaluated genotypes, the traditional aromatic rice Jeerakasala exhibited the highest OsMRP13 expression, showing an approximately 2.2-fold increase relative to the control. The aromatic rice Basmati also displayed elevated expression, with an approximately 1.8-fold increase, although lower than that observed in Jeerakasala. The released variety Pratyasa showed a slight increase in transcript abundance, with a relative fold change of approximately 1.2, indicating only marginal upregulation compared with the control. The expression profile demonstrates that OsMRP13 was upregulated in all the tested rice varieties relative to the control, with the aromatic cultivars exhibiting substantially higher transcript levels than the released varieties. The relatively larger error bars observed for Jeerakasala, Basmati, and Pratyasa indicate moderate biological variation among replicates, whereas the control exhibited consistent expression.

OsMRP13 encodes a member of the multidrug resistance-associated protein (MRP/ABCC) transporter family, which functions in the transport and sequestration of phytic acid into protein storage vacuoles during seed development. The significantly higher expression observed in

Jeerakasala suggests enhanced transport and accumulation of phytic acid within the grain, while the elevated expression in Basmati also indicates active phytic acid sequestration. In contrast, the comparatively lower expression in Pratyasa suggests reduced transporter activity, although expression remained slightly higher than the control.

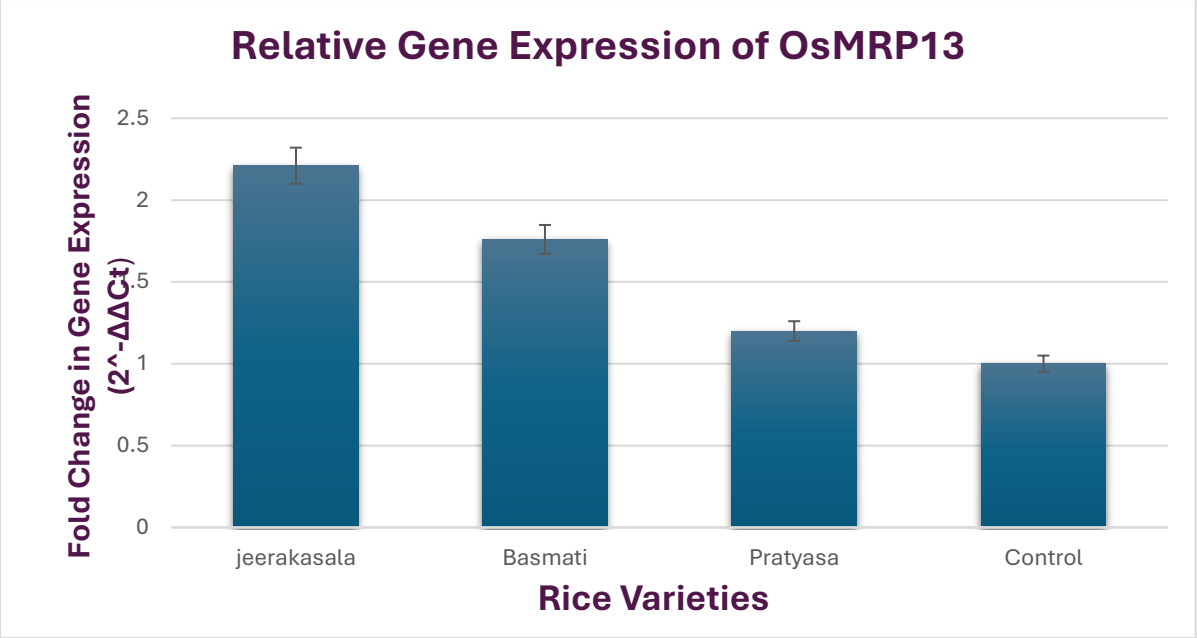


Figure 11: Relative expression analysis of the OsMRP13 gene in selected rice varieties determined by quantitative real-time PCR (qRT-PCR). Relative transcript levels were calculated using the 2^-ΔΔCt method with GAPDH as the internal reference gene and Uma as the calibrator (control; fold change = 1)

Overall, the results indicate that OsMRP13 is differentially regulated among the selected rice genotypes. Both aromatic rice varieties (Jeerakasala and Basmati) exhibited higher expression than the released varieties (Uma and Pratyasa), suggesting that aromatic cultivars possess a greater capacity for phytic acid transport and storage. When considered together with the expression profiles of RINO1, OsMIK, OsITPK1, and OsPGK1, these findings indicate coordinated yet genotype-specific regulation of the phytic acid biosynthetic pathway. The consistently higher expression of several pathway genes in Jeerakasala highlights its enhanced transcriptional activity for phytic acid biosynthesis and accumulation, whereas Basmati exhibited selective upregulation of specific genes. These genotype-dependent differences may contribute to variation in phytic acid content, phosphorus storage, and mineral bioavailability among aromatic and non-aromatic rice varieties.

Chapter 6

Discussion

The present investigation aimed to understand the molecular regulation of the phytic acid biosynthetic pathway in selected rice varieties by analysing the expression of five important genes (OsPGK1, RINO1, OsMIK, OsITPK1 and OsMRP13) using quantitative real-time PCR. Two traditional aromatic rice varieties (Jeerakasala and Basmati) and two released non-aromatic varieties (Pratyasa and Uma) were selected to compare differences in transcriptional regulation associated with phytic acid biosynthesis and phytate transport. High-quality RNA and cDNA were obtained from all genotypes, and the amplification and melt curve analyses confirmed the specificity of all primers used, ensuring reliable gene expression analysis. The qRT-PCR results demonstrated clear genotype-dependent differences in the expression of genes involved in phytic acid metabolism. Although all five genes belong to the same metabolic pathway, each exhibited a distinct expression profile among the studied varieties, indicating that phytic acid biosynthesis is regulated through coordinated but gene-specific mechanisms rather than by uniform expression of the entire pathway. Among the tested genotypes, the traditional aromatic rice Jeerakasala consistently exhibited the highest expression of OsPGK1, OsMIK, OsITPK1 and OsMRP13, whereas Basmati showed the highest expression only for RINO1. In contrast, the released variety Pratyasa generally exhibited lower transcript abundance for most genes, while Uma served as the baseline control.

The marked upregulation of OsPGK1 in Jeerakasala suggests enhanced glycolytic activity associated with phytic acid biosynthesis. Phosphoglycerate kinase provides ATP and metabolic intermediates required for the synthesis of myo-inositol, thereby supporting the formation of phytic acid. Tagashira et al. (2015) demonstrated that overexpression of OsPGK1 increased phytic acid accumulation in rice seeds together with enhanced expression of downstream biosynthetic genes. The approximately 5.5-fold increase observed in Jeerakasala therefore indicates a greater metabolic potential for phytic acid synthesis. Conversely, the reduced expression in Basmati may indicate that this aromatic variety relies less on OsPGK1-mediated regulation or utilises alternative regulatory mechanisms within the pathway. Unlike OsPGK1, RINO1 showed a contrasting expression pattern. Basmati exhibited the highest transcript abundance, whereas Jeerakasala and

Pratyasa showed reduced expression relative to Uma. RINO1 encodes myo-inositol-3-phosphate synthase (MIPS), which catalyses the first committed and rate-limiting step of myo-inositol biosynthesis. Previous studies by Yoshida et al. (1999) and Perera et al. (2019) reported that increased RINO1 expression is closely associated with enhanced phytate accumulation during grain development. Similarly, Kuwano et al. (2006) demonstrated that suppression of RINO1 significantly reduced phytic acid accumulation in rice seeds. Therefore, the higher RINO1 expression observed in Basmati suggests increased synthesis of myo-inositol, whereas the comparatively lower expression in Jeerakasala indicates that downstream enzymes may compensate for reduced RINO1 transcript abundance.

Among all the genes analysed, OsMIK exhibited the greatest fold increase, particularly in Jeerakasala (approximately seven-fold). OsMIK catalyses phosphorylation of myo-inositol, producing intermediates essential for subsequent phytic acid biosynthesis. Elevated expression of OsMIK indicates active conversion of myo-inositol into phosphorylated compounds required for phytate formation. Ali et al. (2013) reported that suppression of myo-inositol biosynthesis reduced expression of downstream phytic acid pathway genes, highlighting the importance of this enzyme in maintaining pathway continuity. Therefore, the exceptionally high OsMIK expression observed in Jeerakasala strongly suggests enhanced metabolic flux through the phytic acid pathway. Similarly, OsITPK1 was upregulated in all experimental varieties, with the highest expression again recorded in Jeerakasala followed by Basmati. OsITPK1 catalyses sequential phosphorylation reactions that convert lower inositol phosphates into highly phosphorylated intermediates preceding phytic acid formation. Previous studies by Suzuki et al. (2007) and Karmakar et al. (2020) demonstrated that ITPK enzymes are essential regulators of phytic acid accumulation during rice seed development. The elevated OsITPK1 expression observed in aromatic rice varieties therefore supports their greater biosynthetic capacity for phytic acid production.The expression pattern of OsMRP13 differed slightly because this gene is involved in phytate transport rather than biosynthesis. OsMRP13 encodes an ATP-binding cassette transporter responsible for transporting phytic acid into vacuoles where it is stored as phytin. Jeerakasala again exhibited the highest expression, followed by Basmati and Pratyasa. Similar observations have been reported by Nagy et al. (2009), Xu et al. (2009) and Shi et al. (2007), who demonstrated that mutations affecting ABCC transporters markedly reduce phytate accumulation despite normal biosynthesis.

Therefore, increased OsMRP13 expression in Jeerakasala indicates enhanced efficiency of phytate transport and storage within developing rice grains.

An important finding of the present study is that aromatic rice varieties did not exhibit identical expression patterns despite belonging to the same grain quality group. Jeerakasala showed coordinated upregulation of four genes involved in phytic acid biosynthesis and transport (OsPGK1, OsMIK, OsITPK1 and OsMRP13), whereas Basmati exhibited particularly high expression only for RINO1. This observation suggests that transcriptional regulation of the phytic acid pathway is genotype-specific rather than being solely determined by aromatic characteristics. Similarly, the released varieties Uma and Pratyasa also differed in gene expression, indicating that breeding history and genetic background may significantly influence phosphorus metabolism. When the expression profiles of all five genes are considered together, Jeerakasala appears to possess the strongest overall molecular machinery for phytic acid biosynthesis and phytate accumulation. High expression of OsPGK1, OsMIK and OsITPK1 indicates efficient biosynthesis of inositol phosphate intermediates, while elevated OsMRP13 expression supports efficient vacuolar transport and storage of phytate. In contrast, Basmati appears to rely primarily on increased RINO1 expression during the initial stage of the pathway, whereas Pratyasa maintained comparatively lower expression of most genes, suggesting reduced metabolic activity related to phytic acid synthesis.

Hence the present study demonstrates that expression of phytic acid pathway genes is strongly influenced by genotype. The coordinated regulation observed in Jeerakasala indicates that this traditional aromatic variety may possess a greater capacity for phytic acid synthesis and phosphorus storage than the released cultivars. These findings agree with previous reports describing the complex genetic regulation of phytic acid metabolism and provide valuable molecular information for rice improvement programmes. Identification of genotypes exhibiting naturally lower expression of key biosynthetic genes may facilitate the development of rice varieties with reduced phytic acid content, thereby improving mineral bioavailability without adversely affecting grain development. Conversely, genes showing consistently high expression may serve as useful molecular markers for understanding phosphorus metabolism and seed nutritional quality in rice.

Summary and Conclusion

Phytic acid is the principal storage form of phosphorus in rice grains which plays an essential role in seed development and mineral storage, although it reduces the bioavailability of important minerals such as iron and zinc. This study evaluated the expression of five key genes involved in phytic acid biosynthesis and transport (OsPGK1, RINO1, OsMIK, OsITPK1, and OsMRP13) in aromatic rice varieties (Jeerakasala and Basmati) and non-aromatic released varieties (Pratyasa and Uma) using quantitative real-time PCR (qRT-PCR). High-Quality RNA isolation followed by cDNA preparation enabled reliable gene expression analysis, which revealed significant genotype-dependent differences among the varieties. Jeerakasala exhibited the highest expression of most genes, indicating a more active phytic acid biosynthetic pathway, whereas Basmati showed predominant expression of RINO1, suggesting variation in the regulation of the initial steps of myo-inositol metabolism. The released varieties, particularly Pratyasa, displayed comparatively lower expression levels, while Uma maintained baseline expression.

The study demonstrates that phytic acid biosynthesis in rice is regulated through genotype-specific transcriptional mechanisms rather than by aromatic or non-aromatic classification alone. The contrasting expression patterns observed between traditional aromatic and released non-aromatic varieties highlight the rich genetic diversity available for improving grain nutritional quality. These findings provide valuable molecular markers for breeding programmes aimed at optimizing phytic acid content, enhancing mineral bioavailability, and developing nutritionally superior rice cultivars while preserving desirable agronomic traits. Furthermore, this work establishes a foundation for future studies integrating gene expression with phytate content, mineral composition, and functional validation of candidate genes.

References

Afify, A. E. M. M. R., El-Beltagi, H. S., Abd El-Salam, S. M., & Omran, A. A. (2011). Bioavailability of iron, zinc, phytate and phytase activity during soaking and germination of white sorghum varieties. *PLoS ONE, 6*(10), e25512.

Al Hasan, S. M., Hassan, M., Saha, S., Islam, M., Billah, M., & Islam, S. (2016). Dietary phytate intake inhibits the bioavailability of iron and calcium in the diets of pregnant women in rural Bangladesh: A cross-sectional study. *BMC Nutrition, 2*, Article 24.

Ali, N., Paul, S., Gayen, D., Sarkar, S. N., Datta, S. K., & Datta, K. (2013). RNAi-mediated down-regulation of *myo*-inositol-3-phosphate synthase to generate low-phytate rice. *Rice, 6*(1), Article 12. https://doi.org/10.1186/1939-8433-6-12

Azeke, M. A., Egielewa, S. J., Eigbogbo, M. U., & Ihimire, I. G. (2011). Effect of germination on the phytase activity, phytate and total phosphorus contents of rice (*Oryza sativa*), maize (*Zea mays*), millet (*Panicum miliaceum*), sorghum (*Sorghum bicolor*), and wheat (*Triticum aestivum*). *Journal of Food Science and Technology, 48*(6), 724–

Azharudheen, T. P. M., Kumar, A., Anilkumar, C., Sah, R. P., Behera, S., & Marndi, B. C. (2022). Understanding natural genetic variation for grain phytic acid content and functional marker development for phytic acid-related genes in rice. *Molecular Biology Reports, 49*(11), 10479–10491. https://doi.org/10.1007/s11033-022-07801-9

Behera, L., Pradhan, S. K., Azharudheen, T. P., Anandan, A., & Roy, S. (2026). Association mapping of grain quality and phytic acid content in aromatic rice using candidate gene-based SSR markers. *Rice Science, 33*(1), 45–58.

Bhattacharjee, P., Singhal, R. S., & Kulkarni, P. R. (2002). Basmati rice: A review. *International Journal of Food Science & Technology, 37*(1), 1–12.

Bisne, R., & Sarawgi, A. K. (2008). Agro-morphological and quality characterization of aromatic rice germplasm. *Bangladesh Journal of Agricultural Research, 33*(3), 479–492.

Bolger, T. A., Folkmann, A. W., Tran, E. J., & Wente, S. R. (2008). The mRNA export factor Gle1 and inositol hexakisphosphate regulate distinct stages of translation. *Cell, 134*(4), 624–633. https://doi.org/10.1016/j.cell.2008.08.023

Bregitzer, P., & Raboy, V. (2006). Effects of four independent low-phytate mutations in barley (*Hordeum vulgare* L.) on seed phosphorus characteristics and malting quality. *Cereal Chemistry, 83*(5), 460–464. https://doi.org/10.1094/CC-83-0460

Burbano, C., Muzquiz, M., Osagie, A., Ayet, G., & Cuadrado, C. (1995). Determination of phytate and lower inositol phosphates in Spanish legumes by HPLC methodology. *Food Chemistry, 52*(3), 321–325. https://doi.org/10.1016/0308-8146(95)92882-P

Bustin, S. A., Benes, V., Garson, J. A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M. W., Shipley, G. L., Vandesompele, J., & Wittwer, C. T. (2009). The MIQE guidelines: Minimum information for publication of quantitative real-time PCR experiments. *Clinical Chemistry, 55*(4), 611–622.

Chan, S. S. L., Ferguson, E. L., Bailey, K., Fahmida, U., Harper, T. B., & Gibson, R. S. (2007). The concentrations of iron, calcium, zinc and phytate in cereals and legumes habitually consumed by infants living in East Lombok, Indonesia. *Journal of Food Composition and Analysis, 20*(7), 609–617. https://doi.org/10.1016/j.jfca.2007.03.003

Cheema, A. A. Z., Ashraf, M., Rashid, M., & Tahir, G. R. (2005). Association analysis of some yield influencing traits in aromatic and non-aromatic rice. *Pakistan Journal of Botany, 37*(3), 613–627.

Cheng, Z. Q., Huang, X. Q., Zhang, Y. Z., & Qian, J. (2005). Diversity in the content of some nutritional components in husked seeds of three wild rice species and rice varieties in Yunnan Province of China. *Journal of Integrative Plant Biology, 47*(10), 1260–1270. https://doi.org/10.1111/j.1744-7909.2005.00140.x

Cominelli, E., Pilu, R., & Sparvoli, F. (2020). Phytic acid and transporters: What can we learn from low-phytic-acid mutants? *Plants, 9*(1), Article 69.

Couzy, F., Mansourian, R., Labate, A., Guinchard, S., Montagne, D. H., & Dirren, H. (1998). Effect of dietary phytic acid on zinc absorption in healthy elderly subjects as assessed by serum zinc

concentration curves. *British Journal of Nutrition, 80*(2), 179–184. https://doi.org/10.1017/S0007114598001293

Desingu, L., Visakh, R. L., Sah, R. P., Jha, U. C., Manju, R. V., Alex, S., & Beena, R. (2025). Genetic regulation of phytic acid biosynthesis in rice: Pathways and breeding approaches for low-phytate varieties. *Rice Science, 32*(6), 797–814.

Doria, E., Galleschi, L., Calucci, L., Pinzino, C., Pilu, R., Cassani, E., & Nielsen, E. (2009). Phytic acid prevents oxidative stress in seeds: Evidence from a maize (*Zea mays* L.) low-phytate mutant. *Journal of Experimental Botany, 60*(3), 967–978. https://doi.org/10.1093/jxb/ern345

Emami, K., Morris, N. J., Cockell, S. J., Golebiowska, G., Shu, Q. Y., & Gatehouse, A. M. R. (2010). Changes in protein expression profiles between a low-phytic-acid rice (*Oryza sativa* L. ssp. *japonica*) line and its parental line: A proteomic and bioinformatic analysis. *Journal of Agricultural and Food Chemistry, 58*(11), 6912–6922. https://doi.org/10.1021/jf100151m

Garcia-Oliveira, A. L., Tan, L., Fu, Y., & Sun, C. (2009). Genetic identification of quantitative trait loci for contents of mineral nutrients in rice grain. *Journal of Integrative Plant Biology, 51*(1), 84–92. https://doi.org/10.1111/j.1744-7909.2008.00730.x

Ghosh, P., & Roychoudhury, A. (2020). Nutrition and antioxidant profiling in the unpolished and polished grains of eleven indigenous aromatic rice cultivars. *Scientific Reports, 10*, Article 548.

Gong, D., Zhang, X., He, F., Li, Y., Wang, J., & Liu, Z. (2023). Genetic improvements in rice grain quality: A review of elite genes and their applications in molecular breeding. *Agronomy, 13*(5), Article 1375. https://doi.org/10.3390/agronomy13051375

Graf, E. (1983). Applications of phytic acid. *Journal of the American Oil Chemists' Society, 60*(11), 1861–1867.\

Hayakawa, T., Suzuki, K., Miura, H., Ohno, T., & Igaue, I. (1990). *Myo*-inositol polyphosphate intermediates in the dephosphorylation of phytic acid by acid phosphatase with phytase activity from rice bran. *Agricultural and Biological Chemistry, 54*(2), 279–286. https://doi.org/10.1080/00021369.1990.10869943

Hu, X., Lu, L., Guo, Z., & Zhu, Z. (2020). Volatile compounds, affecting factors and evaluation methods for rice aroma: A review. *Trends in Food Science & Technology, 97*, 136–146. https://doi.org/10.1016/j.tifs.2020.01.003

Julia, C., Wissuwa, M., Kretzschmar, T., Jeong, K., & Rose, T. (2016). Phosphorus uptake, partitioning and redistribution during grain filling in rice. *Annals of Botany, 118*(6), 1151–1162.

Karmakar, A., Bhattacharya, S., Sengupta, S., Ali, N., Sarkar, S. N., Datta, K., & Datta, S. K. (2020). RNAi-mediated silencing of the *ITPK* gene reduces phytic acid content, alters transcripts of phytic acid biosynthetic genes, and modulates mineral distribution in rice seeds. *Rice Science, 27*(4), 315–328. https://doi.org/10.1016/j.rsci.2020.05.007

Kies, A. K., de Jonge, L. H., Kemme, P. A., & Jongbloed, A. W. (2006). Interaction between protein, phytate, and microbial phytase. *Journal of Agricultural and Food Chemistry, 54*(5), 1753–1758.

Kim, S. I., Andaya, C. B., Goyal, S. S., & Tai, T. H. (2008). The rice *OsLpa1* gene encodes a novel protein involved in phytic acid metabolism. *Theoretical and Applied Genetics, 117*(5), 769–779.

Kumar, A., Dash, G. K., Sahoo, S. K., Lal, M. K., Sahoo, U., Sah, R. P., Ngangkham, U., Kumar, S., Baig, M. J., & Lenka, S. K. (2023). Phytic acid: A reservoir of phosphorus in seeds plays a dynamic role in plant and animal metabolism. *Plant Foods for Human Nutrition, 78*(2), 183–198. https://doi.org/10.1007/s11130-023-01044-6

Kumar, A., Lal, M. K., Sahoo, S. K., Dash, G. K., Sahoo, U., Nayak, L., Bagchi, T. B., Sah, R. P., Behera, S., & Sharma, S. (2023). The diversity of phytic acid content and grain processing play decisive roles in mineral bioavailability in rice. *Journal of Food Composition and Analysis, 115*, Article 105032.

Kumar, A., Sahu, C., Panda, P. A., Biswal, M., Sah, R. P., Lal, M. K., Baig, M. J., Swain, P., Behera, L., Chattopadhyay, K., & Sharma, S. (2020). Phytic acid content may affect starch digestibility and glycemic index value of rice (*Oryza sativa* L.). *Journal of the Science of Food and Agriculture, 100*(4), 1598–1607. https://doi.org/10.1002/jsfa.10163

Kuwano, M., Ohyama, A., Tanaka, Y., Mimura, T., Takaiwa, F., & Yoshida, K. T. (2006). Molecular breeding for transgenic rice with a low-phytic-acid phenotype through manipulating the *myo*-inositol-3-phosphate synthase gene. *Molecular Breeding, 18*(3), 263–272.

Liang, J., Han, B.-Z., Nout, M. J. R., & Hamer, R. J. (2006). Iron, zinc and phytic acid content of selected rice varieties from China and their implications for mineral bioavailability. *Journal of Agricultural and Food Chemistry, 54*(12), 4634–4639. https://doi.org/10.1021/jf060470z

Liu, J. C., Ockenden, I., Truax, M., & Lott, J. N. A. (2004). Phytic acid-phosphorus and other nutritionally important mineral nutrient elements in grains of wild-type and low-phytic-acid (*lpa1-1*) rice. *Seed Science Research, 14*(2), 109–116.

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2^−ΔΔCt method. *Methods, 25*(4), 402–408. https://doi.org/10.1006/meth.2001.1262

Mahender, A., Anandan, A., Pradhan, S. K., & Pandit, E. (2016). Rice grain nutritional traits and their enhancement using relevant genes and QTLs through advanced approaches. *SpringerPlus, 5*, Article 2086. https://doi.org/10.1186/s40064-016-3744-6

McCall, K. A., Huang, C., & Fierke, C. A. (2000). Function and mechanism of zinc metalloenzymes. *The Journal of Nutrition, 130*(5 Suppl.), 1437S–1446S.

Miller, L. V., Krebs, N. F., & Hambidge, K. M. (2007). A mathematical model of zinc absorption in humans as a function of dietary zinc and phytate. *The Journal of Nutrition, 137*(1), 135–141. https://doi.org/10.1093/jn/137.1.135

Mishra, A., Shamim, M., Siddiqui, M. W., Singh, A., Srivastava, D., & Singh, K. N. (2020). Genotypic variation in spatial distribution of Fe in rice grains in relation to phytic acid content and ferritin gene expression. *Rice Science, 27*(3), 227–236. https://doi.org/10.1016/j.rsci.2020.04.005

Mohidem, N. A., Hashim, N., Shamsudin, R., & Che Man, H. (2022). Rice for food security: Revisiting its production, diversity, rice milling process and nutrient content. *Agriculture, 12*(6), Article 741.

Nagy, R., Grob, H., Weder, B., Green, P., Klein, M., Frelet-Barrand, A., Schjoerring, J. K., Brearley, C. A., & Martinoia, E. (2009). The *Arabidopsis* ATP-binding cassette protein AtMRP5/ABCC5 is a

high-affinity inositol hexakisphosphate transporter involved in guard cell signalling and phytate storage. *Journal of Biological Chemistry, 284*(48), 33614–33622.

Perera, I., Fukushima, A., Akabane, T., Horiguchi, G., Seneweera, S., & Hirotsu, N. (2019). Expression regulation of *myo*-inositol 3-phosphate synthase 1 (*INO1*) in determination of phytic acid accumulation in rice grain. *Scientific Reports, 9*, Article 14866. https://doi.org/10.1038/s41598-019-51327-3

Perera, I., Seneweera, S., & Hirotsu, N. (2018). Manipulating the phytic acid content of rice grain toward improving micronutrient bioavailability. *Rice, 11*, Article 4. https://doi.org/10.1186/s12284-018-0200-y

Raboy, V. (1997). Accumulation and storage of phosphate and minerals. In B. A. Larkins & I. K. Vasil (Eds.), *Cellular and molecular biology of plant seed development* (Advances in Cellular and Molecular Biology of Plants, Vol. 4, pp. 441–477). Springer. https://doi.org/10.1007/978-94-015-8909-3_9

Raboy, V. (2000). Low-phytic-acid grains. *Food and Nutrition Bulletin, 21*(4), 423–427. https://doi.org/10.1177/156482650002100416

Raboy, V. (2020). Low phytic acid crops: Observations based on four decades of research. *Plants, 9*(2), Article 140.

Rose, T. J., Pariasca-Tanaka, J., Rose, M. T., Fukuta, Y., & Wissuwa, M. (2010). Genotypic variation in grain phosphorus concentration and opportunities to improve phosphorus-use efficiency in rice. *Field Crops Research, 119*(2–3), 154–160.

Saiardi, A., Sciambi, C., McCaffery, J. M., Wendland, B., & Snyder, S. H. (2002). Inositol pyrophosphates regulate endocytic trafficking. *Proceedings of the National Academy of Sciences, 99*(22), 14206–14211. https://doi.org/10.1073/pnas.212527899

Sakai, H., Iwai, T., Matsubara, C., Usui, Y., Okamura, M., Yatou, O., Terada, Y., Aoki, N., Nishida, S., & Yoshida, K. T. (2015). A decrease in phytic acid content substantially affects the distribution of mineral elements within rice seeds. *Plant Science, 238*, 170–177. https://doi.org/10.1016/j.plantsci.2015.06.015

Sandberg, A.-S., Brune, M., Carlsson, N.-G., Hallberg, L., Skoglund, E., & Rossander-Hultén, L. (1999). Inositol phosphates with different numbers of phosphate groups influence iron absorption in humans. *The American Journal of Clinical Nutrition, 70*(2), 240–246. https://doi.org/10.1093/ajcn/70.2.240

Sarla, N., Swamy, B. P. M., Kaladhar, K., Anuradha, K., Rao, V. Y., Batchu, A. K., Agarwal, S., Babu, A. P., Sudhakar, T., Sreenu, K., Longvah, T., Surekha, K., Rao, K. V., Reddy, G. A., Roja, T. V., Kiranmayi, S. L., Radhika, K., Manorama, K., Cheralu, C., & Viraktamath, B. C. (2012). Increasing iron and zinc in rice grains using deep-water rices and wild species: Identifying genomic segments and candidate genes. *Quality Assurance and Safety of Crops & Foods, 4*(3), 138–149.

Shi, J., Wang, H., Hazebroek, J., Ertl, D. S., & Harp, T. (2007). The maize low-phytic-acid mutant *lpa1* is caused by mutation in an inositol phosphate transporter gene (*ZmMRP4*). *Plant Physiology, 144*(1), 561–573. https://doi.org/10.1104/pp.107.096909

Sparvoli, F., & Cominelli, E. (2015). Seed biofortification and phytic acid reduction: A conflict of interest for the plant? *Plants, 4*(4), 728–755. https://doi.org/10.3390/plants4040728

Suzuki, M., Tanaka, K., Kuwano, M., & Yoshida, K. T. (2007). Expression pattern of inositol phosphate-related enzymes in rice (*Oryza sativa* L.): Implications for the phytic acid biosynthetic pathway. *Gene, 405*(1–2), 55–64. https://doi.org/10.1016/j.gene.2007.08.019

Tagashira, Y., Shimizu, T., Miyamoto, M., Nishida, S., & Yoshida, K. T. (2015). Overexpression of a gene involved in phytic acid biosynthesis substantially increases phytic acid and total phosphorus in rice seeds. *Plants, 4*(2), 196–208. https://doi.org/10.3390/plants4020196

Torabinejad, J., Donahue, J. L., Gunesekera, B. M., Allen-Daniels, M. J., & Gillaspy, G. E. (2009). VTC4 is a bifunctional enzyme that affects *myo*-inositol and ascorbate biosynthesis in plants. *Plant Physiology, 150*(2), 951–961. https://doi.org/10.1104/pp.109.138263

Vanishri, B. R. (2016). *Molecular marker diversity of micronutrients and grain oil content in aromatic and non-aromatic rice (Oryza sativa L.) varieties* (Master's thesis). University of Agricultural Sciences, Bengaluru, India.

Wang, K. M., Wu, J. G., Li, G., Zhang, D. P., Yang, Z. W., & Shi, C. H. (2011). Distribution of phytic acid and mineral elements in three *indica* rice (*Oryza sativa* L.) cultivars. *Journal of Cereal Science, 54*(1), 116–121. https://doi.org/10.1016/j.jcs.2011.04.006

Wang, R., & Guo, S. (2021). Phytic acid and its interactions: Contributions to protein functionality, food processing, and safety. *Comprehensive Reviews in Food Science and Food Safety, 20*(2), 2081–2105. https://doi.org/10.1111/1541-4337.12714

Wilson, M. S., Bulley, S. J., Pisani, F., Irvine, R. F., & Saiardi, A. (2015). A novel method for the purification of inositol phosphates from biological samples reveals that no phytate is present in human plasma or urine. *Open Biology, 5*(3), Article 150014. https://doi.org/10.1098/rsob.150014

Xu, X. H., Zhao, H. J., Liu, Q. L., Frank, T., Engel, K. H., An, G., Shu, Q. Y., & Wang, C. R. (2009). Mutations of the multidrug resistance-associated protein ABC transporter gene *OsABCC13* are responsible for the rice low phytic acid 1 phenotype. *Plant Physiology, 151*(1), 171–181. https://doi.org/10.1104/pp.109.141788

Yoshida, K. T., Wada, T., Koyama, H., Mizobuchi-Fukuoka, R., & Naito, S. (1999). Temporal and spatial patterns of accumulation of the transcript of *myo*-inositol-1-phosphate synthase and phytin-containing particles during seed development in rice. *Plant Physiology, 119*(1), 65–72. https://doi.org/10.1104/pp.119.1.65

Zhao, H. J., Liu, Q. L., Ren, X. L., Wu, D. X., & Shu, Q. Y. (2008). Gene identification and allele-specific marker development for two low phytic acid mutations in rice (*Oryza sativa* L.). *Molecular Breeding, 22*(4), 603–612. https://doi.org/10.1007/s11032-008-9197-2

Zhou, J. R., & Erdman, J. W., Jr. (1995). Phytic acid in health and disease. *Critical Reviews in Food Science and Nutrition, 35*(6), 495–508. https://doi.org/10.1080/10408399509527712