Determination of Phosphorous-deficient Tolerance in Thai Lowland Rice Cultivars Based on *Pup1*-K46 Marker

Bophal Sok

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Biology (International Program)

Prince of Songkla University

2019

Determination of Phosphorous-deficient Tolerance in Thai Lowland Rice Cultivars Based on *Pup1*-K46 Marker

Bophal Sok

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Biology (International Program)

Prince of Songkla University

2019

Thesis Title	Determination of Phosphorous-deficient Tolerance in Thai Lowland Rice Cultivars Based on *Pup1*-K46 Marker
Author	Miss Bophal Sok
Major Program	Biology (International Program)

Major Advisor

……..……..……...…

(Assoc. Prof. Dr. Upatham Meesawat)

Examining Committee:

….……..……..…Chairperson

(Assoc. Prof. Dr. Duangporn Premjet)

……..…….……...Committee

(Assoc. Prof. Dr. Upatham Meesawat)

…….……...…..Committee

(Asst. Prof. Dr. Krittika Kaewchumnong)

The Graduate School, Prince of Songkla University, has approved this thesis as partial fulfillment of the requirements for the of Master of Science Degree in Biology (International Program)

……..

(Prof. Dr. Damrongsak Faroongsarng)

Dean of Graduate School

This is to certify that the work here submitted is the result of the candidate's own investigations. Due acknowledgement has been made of any assistance received.

……..…….. Signature
(Assoc. Prof. Dr. Upatham Meesawat)
Major Advisor

……..… Signature
(Miss Bophal Sok)
Candidate

I hereby certify that this work has not been accepted in substance for any degree, and is not being currently submitted in candidature for any degree.

……. Signature
(Miss Bophal Sok)
Candidate

Thesis Title Determination of Phosphorous-deficient Tolerance in Thai Lowland Rice Cultivars Based on *Pup1*-K46 Marker

Author Miss Bophal Sok

Major Program Biology (International Program)

Academic Year 2018

ABSTRACT

Phosphorus (P) deficiency is one of the major constraints for rice production due to its natural availability. The low P stress affects rice growth and yield, thereby food security. Using genetic markers to determine P-deficient tolerance is a promising tool to improve rice for sustainable production. *Phosphate uptake 1* (*Pup1*-K46) is a diagnostic gene-based marker to confer the P-deficient tolerance in rice. This marker is dominantly conserved in upland ecotypes, but its presence in lowland rice cultivars has not yet been well-understood. In this study, thirty-one of the 61 Thai lowland rice cultivars were detected with the *Pup1* region. Twenty to 21 lowland rice cultivars with and without the *Pup1*-K46 region were then selected and grown under half- or full-strength low P (0.25 or 0.5 mg/l) and high P (5 or 10 mg/l) Yoshida solutions for three or four weeks to determine the P-deficient tolerance, respectively. Results showed that the low P (LP) condition reduced rice biomass, total P concentration, P uptake, and Pi content in the rice tissues, except for chlorophyll content. Interestingly, the *Pup1*-K46^{+} cultivars maintained less shoot mass reduction and higher relative efficiency of P use (REP) than the *Pup1*-K46^{-} cultivars under both LP conditions. Additionally, the *Pup1*-K46^{+} cultivars accumulated higher root P concentration and P uptake as well as greater amount of Pi content in their shoot and root than the *Pup1*-K46^{-} cultivars. These suggested that the *Pup1*-K46^{+} cultivars were more tolerant to the P starvation by holding superior shoot growth and accumulating higher P uptake and total P concentration via their roots. The *Pup1*-K46^{+} cultivars also maintained soluble Pi against low P availability by storing it in cytoplasm. It is therefore confirmed that the *Pup1*-K46 region empowers lowland rice cultivars to withstand the P deficiency like the upland rice ecotype.

Keywords:

Phosphorous-deficient tolerance, lowland rice, *Pup1*-K46

ACKNOWLEDGEMENTS

With deepest gratitude, I would like to express my heartfelt thanks to my academic supervisor, Assoc. Prof. Dr. Upatham Meesawat, Department of Biology, Faculty of Science, Prince of Songkhla University (PSU), Thailand, for offering valuable advice, comment, and guidance to this work as well as being a committee.

I extend my deepest gratitude to Assoc. Prof. Dr. Duangporn Premjet, Department of Agricultural Science, Faculty of Agriculture Natural Resource and Environment, Naresuan University (NU), Thailand, for giving me invaluable advice, comments and corrections to this work as a chairperson.

I am very thankful to Asst. Prof. Dr. Krittika Kaewchumnong, Department of Biology, Faculty of Science, PSU, for giving the good comments and as a committee.

Special gratitude and thanks to Dr. Lompong Klinnawee, Department of Biology, Faculty of Science, PSU, Thailand, for leading me to conduct this research with his helpful advice and guidance, constructive comments and wholehearted support throughout this work. This thesis cannot be completed without his assistance.

I would like to thank Asst. Prof. Dr. Saowapa Duangpan, Department of Plant Science, Faculty of Natural Resources, PSU, for providing the rice seeds.

I wish to express my thanks to Phattalung Rice Research Center, Phattalung, Thailand, and Department of Agriculture, Bangkok, Thailand, for providing the rice seeds to my research.

I would like to thank all the members of Plant Physiology Research Unit from Department Biology, Faculty of Science, PSU, for all their help and sharing criterial experiment for my research.

I am sincerely obliged to the Royal Initiative of Her Royal Highness Princess Maha Chakri Sirindhorn for granting the 2-year scholarship in PSU.

I wish to thank the PSU Graduate School for offering the Graduate Scholarship Grant for conducting my experiments.

Last but not least, I finally owe my loving thanks to my parents, all Cambodia friends and students, seniors in the Laboratory of Plant Tissue Culture, and Mr. Phoura Y for suggestions and encouragements during this overseas study.

Bophal Sok

CONTENTS

	Page
ABSTRACT	v
ACKNOWLEDGEMENTS	vi
CONTENTS	vii
LIST OF TABLES	ix
LIST OF FIGURES	x
LIST OF ABBREVIATIONS	xii
CHAPTER 1 INTRODUCTION	1
1.1 Introduction	1
1.2 Literature review	2
1.2.1 Characteristics of lowland rice (*Oryza sativa* subsp. *indica*)	2
1.2.2 Effects of P deficiency on rice growth and development	4
1.2.3 *Pup1*-K46 as a diagnostic marker for *PSTOL1* gene to confer P-deficient tolerance	8
1.3 Research purpose	10
CHAPTER 2 RESEARCH METHODS	11
2.1 Rice cultivars and seed collection	12
2.2 Screening of the *Pup1*-K46 locus variation	12
2.3 Plant growth conditions	12
2.4 Plant measurements	14
2.4.1 Plant biomass	14
2.4.2 Total P concentration	14
2.4.3 Pi content and Pi recovery	15
2.4.4 Pigment content	18
2.5 Statistical analysis	19
CHAPTER 3 RESULTS	20
3.1 Investigation of *Pup1*-K46 in 61 lowland rice cultivars	20
3.2 Preliminary determination of growth responses of the *Pup1*-K46 positive and *Pup1*-K46 negative cultivars to low P availability in half-strength solution	22
3.2.1 Effects of P deficiency on the rice biomass	22
3.2.2 Determination of P-deficient tolerance by P accumulation and PUE	25

3.2.3 Effect of P deficiency on chlorophyll contents in rice leaves by chlorophyll meter 27
3.3 Determination of growth responses of the *Pup1*-K46 positive and *Pup1*-K46 negative cultivars to low P availability in full-strength solution 28
3.3.1 Effects of P deficiency on the rice biomass 28
3.3.2 Total P concentration under P deficiency 30
3.3.3 P uptake under P deficiency 30
3.3.4 PUE under P deficiency 31
3.3.5 Relationship between P uptake and PUE under P deficiency 34
3.3.6 Pi content under P deficiency 36
3.3.7 Pi recovery efficiency after P deficiency 38
3.3.8 Pigment content changes due to P deficiency 38
3.3.9 Relationship between Pi content and pigment content 40
CHAPTER 4 DISCUSSION 42
4.1 The *Pup1*-K46 marker is detected in half of the lowland rice cultivars in southern Thailand 42
4.2 The *Pup1*-K46 positive cultivars are more tolerant under P deficiency than the *Pup1*-K46 negative cultivars 42
4.3 The P-deficient tolerance of the *Pup1*-K46 positive cultivars are involved with P uptake and Pi content 43
4.3.1 The *Pup1*-K46 positive cultivars accumulate more P in root than the *Pup1*-K46 negative cultivars under P deficiency 43
4.3.2 The *Pup1*-K46 positive cultivars deposited more Pi than the *Pup1*-K46 negative cultivars under P deficiency 45
CHAPTER 5 CONCLUSION 47
REFERENCES 48
APPENDICES 56
Appendix A 57
Appendix B 58
Appendix C 60
Appendix D 71

LIST OF TABLES

Tables		Page
Table 2.1	The prepared standard curve to calculate Pi content	17
Table S1	List of *Pup1*-K46^{+} and *Pup1*-K46^{-} cultivars used in the preliminary experiment and the main experiments	57
Table S2	Preparation of stock solution and components for the half-strength Yoshida solution	58
Table S3	Preparation of stock solution and components for the full-strength Yoshida solution	59
Table S4	Biomass of the *Pup1*-K46^{+} and *Pup1*-K46^{-} rice cultivars under half-strength Yoshida solution	60
Table S5	SPAD values measured by chlorophyll meter on rice leaves of *Pup1*-K46^{+} and *Pup1*-K46^{-} cultivars under half-strength Yoshida solution	62
Table S6	Biomass of the *Pup1*-K46^{+} and *Pup1*-K46^{-}rice cultivars in full-strength Yoshida solution	63
Table S7	P concentration of the *Pup1*-K46^{+} and *Pup1*-K46^{-} rice cultivars in the root and shoot under full-strength Yoshida solution	65
Table S8	P uptake of the *Pup1*-K46^{+} and *Pup1*-K46^{-} cultivars in the shoot and root under full-strength Yoshida solution	66
Table S9	PUE of the *Pup1*-K46^{+} and *Pup1*-K46^{-} cultivars in the shoot and root under full-strength Yoshida solution	67
Table S10	Pi content of the *Pup1*-K46^{+} and *Pup1*-K46^{-} cultivars under the HP, LP and PR in the shoot and root at full-strength Yoshida solution	68

LIST OF FIGURES

Figures		Page
Figure 1.1	Morphology of a rice plant	3
Figure 1.2	Symptoms of P deficiency in rice	5
Figure 1.3	P availability in the soil	5
Figure 1.4	Location of the *Pup1*-K46 on rice chromosome 12	9
Figure 2.1	Overview of methodology in this research	11
Figure 2.2	Experimental design of rice grown under P deficiency	13
Figure 2.3	Schematic diagram of the PR experiment	15
Figure 2.4	Pi content standard curve for Pi content test in rice tissue	17
Figure 3.1	Genotyping analysis of the *Pup1*-K46 marker in Thai lowland rice cultivars	21
Figure 3.2	Genotyping analysis of *EF1α* marker in Thai lowland rice cultivars	22
Figure 3.3	Rice seedlings grown under the half-strength solution	23
Figure 3.4	Percentages of dry mass reduction under half-strength solution	24
Figure 3.5	Relative efficiency of P use (REP) under half-strength solution	24
Figure 3.6	Determination of dry weight, total P concentration and PUE under half-strength solution	27
Figure 3.7	SPAD values in the rice leaves under half-strength solution	27
Figure 3.8	Rice seedlings grown under full-strength solution	28
Figure 3.9	The percentage of dry mass reduction under full-strength solution	29
Figure 3.10	Relative efficiency of P use (REP) under full-strength solution	30
Figure 3.11	Determination of total P concentration, P uptake and PUE under full-strength solution	33
Figure 3.12	Correlations between P uptake and PUE in shoot and root of the *Pup1*-K46^{+} and *Pup1*-K46^{-} cultivars under the HP and LP conditions	35
Figure 3.13	Determination of Pi content, translocation factor and Pi recovery efficiency under full-strength solution	37
Figure 3.14	Pigment contents in the rice leaves under full-strength solution	39

Figure 3.15	Correlations between the Pi content and pigment content in the *Pup1*-K46^{+} and *Pup1*-K46^{-} cultivars under the HP and LP conditions	41
Figure S1	Correlation between PUE and biomass of the shoot and root in the *Pup1*-K46^{+} and *Pup1*-K46^{-} cultivars under the HP and LP condition at full-strength Yoshida solution	69
Figure S2	The Pi reduction of the *Pup1*-K46^{+} and *Pup1*-K46^{-} cultivars in the shoot and root under full-strength Yoshida solution	70

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
ATP	Adenosine triphosphate
Chl	Chlorophyll
Chl a	Chlorophyll a
Chl b	Chlorophyll b
d	Day
DM	Dry mass
DNA	Deoxyribonucleic acid
FW	Fresh weight
g	Gram
h	Hour
H_2O_2	Hydrogen peroxide
HP	High phosphorus
Kg	Kilogram
l	Liter
LP	Low phosphorus
LSD	Least significant difference
mg	Milligram
ml	Milliliter
NILs	Near-isogenic lines
P	Phosphorus
PCR	Polymerized chain reaction
Pi	Phosphate
PR	Phosphorus resupply
PSTOL1	*Phosphorus-starvation tolerance 1*
PUE	Phosphorus use efficiency
Pup1	*Phosphorus uptake 1*
Pup1-K46$^+$	*Pup1*-K46 positive
Pup1-K46$^-$	*Pup1*-K46 negative
QTL	Quantitative trait locus
REP	Relative efficiency of phosphorus
RNA	Ribonucleic acid
SPAD	Soil-plant analysis development

CHAPTER 1
INTRODUCTION

1.1 Introduction

Phosphorus (P) deficiency is a plant disorder involved with inadequate P supply and probably the most nutrient-deficient limitation for production of rice, a staple food of more than half of the world's population. P contents less than 3 mg/l in the soil have been classified as P deficiency (Saleque et al., 1998; Yi et al., 2005; Shi et al., 2014). The deficient phenomenon frequently occurs in the paddy fields (Hopkins, 2015) although a large amount of P fertilizer is applied (Nagumo et al., 2013; Yan et al., 2016). This is because of the large proportion of P is naturally in the inaccessible forms (Lan et al., 2012), while less than 15% is present as soluble phosphate (Pi) available to plants (Uwasawa et al., 1988; Phimsirikul & Matoh, 2003; Johri et al., 2015).

P deficiency reduces rice growth by limiting photosynthetic rate (Nanamori et al., 2004; Wissuwa et al., 2005; Yong-fu et al., 2006) as well as chlorophyll contents in the leaves (Xu et al., 2007; Guo et al., 2012). Under P deficiency, photosynthesis is inhibited mainly via reduced photosynthetic intermediates in the Calvin cycle and inadequate energy production. P deprivation also reduces cellular Pi content in rice tissues, which could be reversed by P resupply (Secco et al., 2013; 2015), indicating that external P fertilizers are frequently needed to maintain the internal Pi. To morpho-physiologically adapt to P shortage, the tolerant rice cultivars develop numerous responses, which mainly increase root to shoot ratio (Wissuwa et al., 2005), root surface area (Gamuyao et al., 2012), root hair length and density (Vejchasarn et al., 2016), P uptake and translocation in the whole plant (Liu et al., 2011). These are to ensure P uptake and utilization efficiency, which constitute for the internal P use efficiency (PUE) (Reich et al., 2014).

Determining P-deficient tolerance in rice using gene-based markers is a promising tool for rice improvement under P deficiency. *Phosphorous uptake 1* (*Pup1)* has so far been a renowned quantitative trait locus (QTL) (Wissuwa, et al., 1998; 2002), which offers rice to tolerate P deficiency by improving its P uptake under P starvation (Wissuwa et al., 2001; Chin et al., 2011). Among the *Pup1* markers, *Pup1*-K46 is a diagnostic gene-based marker to confer the P-deficient tolerance in rice (Chin et al.,

2010; 2011; Gamuyao et al., 2012). *Pup1*-K46 is used as the marker to express a serine/threonine receptor-like protein kinase of which its gene is later termed as *phosphorus-starvation tolerance 1* (*PSTOL1*), which is upregulated under P deficiency and expressed in the crown root meristem (Gamuyao et al., 2012). *Pup1*-K46 marker is highly conserved in the P-deficient tolerant rice, mainly in upland ecotype or drought-tolerant cultivars (Chin et al., 2011), but its presence in lowland rice cultivars has not yet been well understood. Thus, this study aimed to diagnose 61 lowland rice cultivars, mainly originated from Southern Thailand using the *Pup1*-K46 marker and clarify their responses under P deficiency. We hypothesized that the lowland rice cultivars with the *Pup1*-K46 locus have better growth performances under P starvation. This research would mainly contribute to the application of the *Pup1* marker for screening lowland rice cultivars tolerant to low P environments, requiring fewer fertilizer inputs while attaining better growth for sustainable rice production.

1.2 Literature review

1.2.1 Characteristics of lowland rice (*Oryza sativa* subsp. *indica*)

Asian rice (*Oryza sativa* L.) is a plant species belonging to the Gramineae (Poaceae) family of grasses, which is an important staple food for about half of the world's population. *Oryza sativa* consists of two main subspecies; *indica,* the non-sticky, long grained, and *japonica*, the sticky, short-grained. *Japonica* cultivars are normally grown in temperate east Asia and upland areas of south and southeast Asia, while *indica* cultivars are mainly lowland rice, grown in mostly flooded areas across tropical Asia including Cambodia and Thailand (Maclean et al., 2013).

Rice is an annual monocarpic grass that once flowers, sets seeds and then dies. Its growth duration lasts 3-6 months, based on cultivars and environmental conditions. The growth can be categorized into three phases: vegetative, reproductive, and ripening. A rice plant is divided into two systems: shoot and root. The shoot system consists of stem (culm), leaf, flower and grain, and the root system is fibrous, occupied by nodal (adventitious or crown) roots (Fig 1.1). Each round hallow stem is composed of a series of nodes and internodes, in which each upper node produces a flat leaf and a bud, which grows into a tiller. At maturity, the plant has a main stem and several tillers, of which each fertile tiller produces a terminal panicle with many single flowered spikelets

developed into grains when ripening (Maclean et al., 2013).

Rice is a semiaquatic plant. It can grow in a wide range of environments or agroecosystems, which can be classified based on altitude (upland vs. lowland) and water availability (irrigated or rainfed) into irrigated lowland rice, rainfed lowland rice and rainfed upland rice. Worldwide, irrigated lowland rice contains about 80 million hectares and provides 75% of the global rice production, while rainfed lowland rice and rainfed upland rice occupy about 52 and 15 million hectares, and supply about 19% and 4% of the world's rice production, respectively (Maclean et al., 2013).

The lowland rice can be explained by the sum of those with a reliable and controlled external supply of water and a drainage system as irrigated rice, and those depending solely on rainfall and runoff as rainfed rice (Zeigler & Puckridge, 1995). Rainfed lowland rice is always subjected to many abiotic stresses such as drought, flood and nutrient deficiency because of their root system limitations to uptake water and nutrients from soils to sustain and improve its growth and yield (Lan et al., 2012 ; Kant et al., 2018). Compared to upland rice, as a trend, lowland rice has a smaller root system with shallower, thinner roots (O'Toole & Bland, 1987; Kondo et al., 2003).

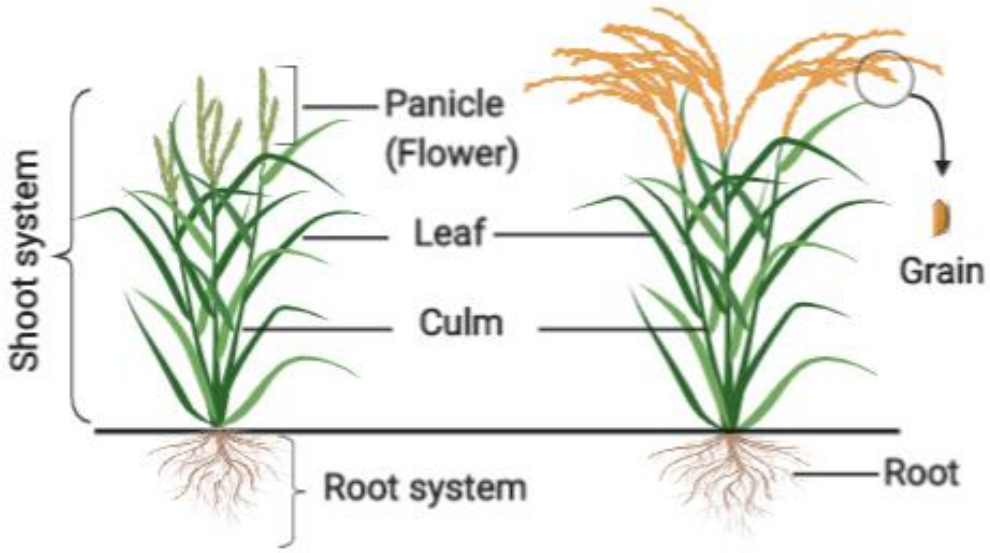


Figure 1.1 Morphology of a rice plant. A plant can be divided into shoot and root systems. The shoot system consists of culm, leaf, flower and grain, while the root system is fibrous, characterized by nodal roots emerged from stem nodes. Image designed by Bophal Sok.

1.2.2 Effects of P deficiency on rice growth and development

1.2.2.1 P deficiency as a major limiting factor for rice production

P is an essential macronutrient for plant growth and development as it is found in adenosine triphosphate (ATP), nucleotides, nucleic acids (DNA, RNA) and phospholipids working in energy storage and transfer and cell membrane integrity. P promotes strong early plant growth and development of a strong root system. It promotes rice tillering, root development, early flowering, and ripening. In paddy fields, the application of mineral P fertilizer is needed during the rice root system establishment and farmers usually apply it as basal or at the first stage of the growing season (Dobermann, 2000).

P concentration less than 3 mg/l in the soil is considered as P deficiency in rice production (Saleque et al., 1998; Yi et al., 2005; Shi et al., 2014). P-deficient rice is stunted, has dirty-dark green to purple coloration of leaves due to increased synthesis of anthocyanin, curly, erect leaves, fewer tillers, and decreased root mass (Fig 1.2) (Dobermann, 2000). P deficiency occurs in all major rice ecosystems. It appears in acid upland soils with high P-fixation capacity, rainfed lowland with coarse-textured soils of less organic matter and P reserves such as sandy soils in northeast Thailand and Cambodia and degraded lowland soils in north Vietnam (Dobermann, 2000). It is also reported as fairly common in irrigated rice although P fertilizer is properly applied (Lan et al., 2012; Yan et al., 2016). One reason is because in natural soils, approximately 85-90% of the total P contents in organic and precipitated forms are unavailable for plants, while only 10 to 15% of the total P is present as soluble phosphate (Pi) ($H_2PO_4^-$ and HPO_4^{2-}) that plants can absorb (Johri et al., 2015) (Fig 1.3). Another reason is because of the highly competitive Pi consumption in the crop fields (Hopkins, 2015).

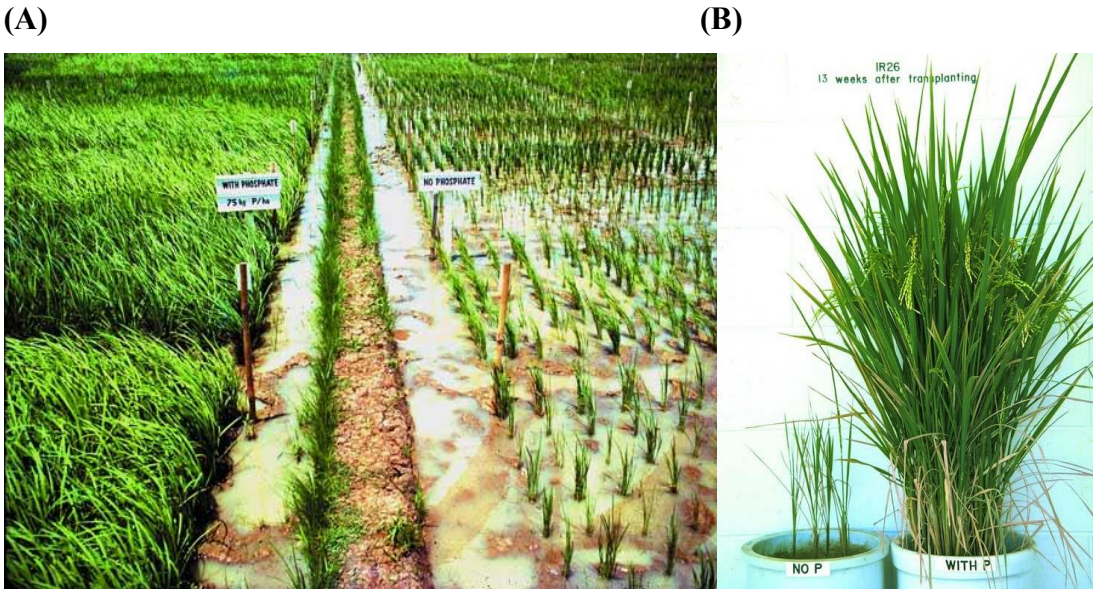


Figure 1.2 Symptoms of P deficiency in rice. (A) At the field and (B) in the pot, rice plants are small, stunted under P deficiency compared to those under normal condition. (Source: IRRI, 2019).

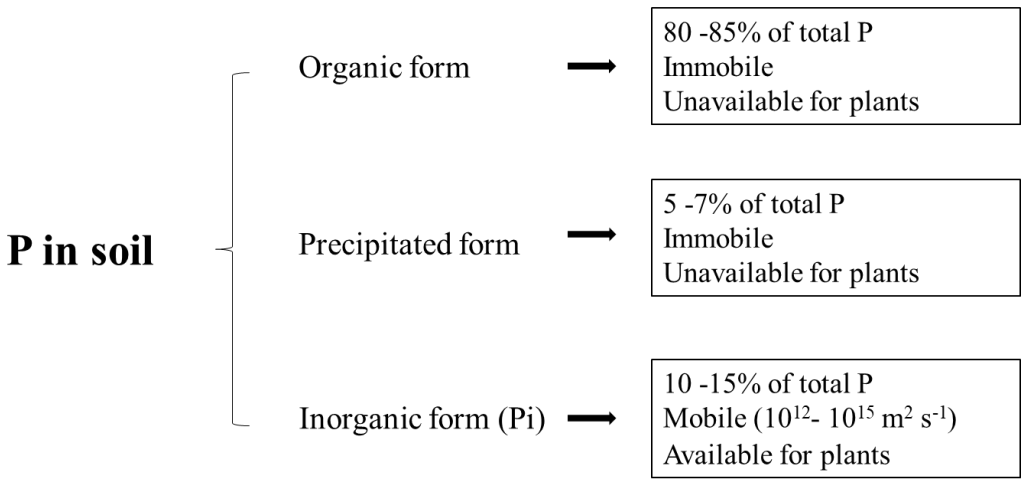


Figure 1.3 P availability in the soil. There are three forms of total P in the soil: organic (80-85%), precipitated (5-7%) and inorganic (Pi; 10-15%) available for plant absorption (Johri et al., 2015).

1.2.2.2 Effects of P deficiency on photosynthetic tissues

P deficiency impairs plant growth especially in photosynthetic tissue by reducing photosynthesis efficiency (Dietz & Foyer, 1986; Rodríguez et al., 1998; Wissuwa et al., 2005; Singh et al., 2018). Under P deficiency, photosynthesis is mainly constrained via reduced photosynthetic phosphorylated intermediates in the Calvin cycle, with inadequate ATP production. For example, one molecule Pi is fit for every three molecules of CO_2 to make trios-Pi in carbon fixation aided by phosphorylated intermediates, so reduced Pi concentration in the leaf adversely affects the Calvin cycle, thereby affecting the photosynthetic rate (Hernández & Munné-Bosch, 2015; Pieters et al., 2001). Reduced photosynthetic rate under P shortage has previously been reported in crops such as barley and spinach (Dietz & Foyer, 1986), soybean (Singh et al., 2018) and wheat (Rodríguez et al., 1998). When the photosynthetic rate was reduced, shoot biomass production was also reduced (Rodríguez et al., 1998; Singh et al., 2018).

Like other crop species, rice also has reduced photosynthetic rate under P deficiency (Nanamori et al., 2004; Wissuwa et al., 2005; Yong-fu et al., 2006). P deficiency reduces leaf dry mass and enzymes required for glycolysis, leading to excessive buildup of carbohydrate in the leaf (Nanamori et al., 2004), and therefore changing pigment content in the leaf. P deficiency also decreased leaf area and net photosynthesis (the rate of CO_2 uptake in photosynthesis minus the rate of CO_2 loss in respiration) by 37% (Wissuwa et al., 2005). It is also reported that P deficiency reduces the total chlorophyll contents, in which content of chlorophyll a is more reduced than that of chlorophyll b (Xu et al., 2007; Guo et al., 2012).

1.2.2.3 Rice response to low P availability by enhancing Pi acquisition strategies

Under the P-starved conditions, plants develop multiple morphological and physiological responses mainly in roots to acquire P from the soil and translocate it to the whole plant body. Such responses mainly include an increase in root to shoot ratio, root surface area, root hair length and density, and an enhance in P uptake, P translocation within the plant, P retention in the roots, and P use efficiency (PUE) (Raghothama, 1999). A typical response to P shortage is the enhanced lateral root growth and branching which was reported in bean (Lynch et al., 2001) and Arabidopsis (Ticconi et al., 2004).

Compared to Arabidopsis as a dicot, response to P deficiency in rice is more complex, firstly probably because their seeds contain more P reserve. Another reason is their fibrous root system. Rice root architecture is composed of primary and seminal roots generated during the embryo development, post-embryonic nodal roots emerged from each stem node, and lateral roots and root hairs bearing from each root type (Morita et al., 1995; Rebouillat et al., 2009). Primary and seminal roots play important roles at the seedling stage, while adventitious roots dominantly function in mature plants after that (Hochholdinger et al., 2004; Rebouillat et al., 2009).

Under P deficiency, rice allocates more carbohydrates to their roots, which probably contributes to the increase of root to shoot dry mass ratio (Wissuwa et al., 2005). Moreover, as the bioavailability of P is in general greatest at topsoil layer (Lynch, 2019), rice produces greater root surface area to forage more P at minimal cost (Gamuyao et al., 2012). In response to P starvation, Vejchasarn et al. (2016) found that all genotypes under P deprivation increased root hair length and density, which is previously reported that up to 90% of total P acquired by plant is absorbed by root hairs (Raghothama, 1999).

Like other plants, rice enhances P uptake, translocation, retention in the roots, and increased PUE under P deficiency. Wissuwa et al. (1998) found that the tolerant rice cultivars could uptake more P than the intolerant rice cultivars under the P-starved condition. When Pi is deprived, rice enhances the efficiency of P uptake and translocation through plasma membrane Pi transporters (Liu et al., 2011). Under P deficiency, by remobilizing it from old shoot tissues, P concentration is more allocated to roots in order to develop its growth to uptake more P and translocate it back to the shoot (Raghothama, 1999). Also, rice attempts to hold the optimum level of inorganic P concentration in their root and shoot tissues; it takes one to three days to reduce P concentration in root and shoot of Nipponbare seedlings after P removal from the culture while it takes a day to recover the decreased Pi level in shoot and root tissues after P resupply (Secco et al., 2013; 2015).

These strategies aim to ensure the efficiency of P uptake and utilization, which constitutes for PUE (Reich et al., 2014). PUE is defined as the amount of biomass produced per unit P; in other words, PUE is the inverse of tissue P concentration. In plant improvement, PUE is an indicator to evaluate P deficiency tolerance in several

plant species (van de Wiel et al., 2016) including rice (Fageria & Santos, 2002; Fageria, 2014; Wang et al., 2014). The higher the PUE the rice can hold, the higher tolerance the rice can have. However, increasing PUE can be achieved by increasing uptake capacity or by optimizing its utilization (Neto et al., 2016).

1.2.3 *Pup1*-K46 as a diagnostic marker for *PSTOL1* gene to confer P-deficient tolerance

As P-deficient tolerance is a challenging trait, improving rice cultivars with this trait is a cost-effective and sustainable manner toward rice production. One of the most successful genetic approach to mitigate P-deficient stress to date is the identification and characterization of *Phosphate uptake 1* (*Pup1*) quantitative trait locus (QTL) on rice chromosome 12 since 1998 (Wissuwa et al., 1998). *Pup1* QTL could confer tolerance to P starvation in rice by improving its P acquisition. This genomic region was initially identified from Kasalath, an *indica* tolerant cultivar from India, and transferred to Nipponbare, a *japonica* intolerant cultivar from Japan, by three backcrosses (Wissuwa & Ae, 2001; Wissuwa et al., 2002). Compared to the parental background Nipponbare, a near-isogenic line (i.e. NIL-C443) carrying *Pup1* could significantly increase greater P uptake and yields of grain and biomass under P deprivation (Wissuwa & Ae., 2001; Wissuwa et al., 2002; 2005).

In order to determine a specific gene/s responsible for the physiological mechanism, DNA sequence of *Pup1* locus in Kasalath was compared to that of Nipponbare (Heuer et al., 2009). The *Pup1* region in Kasalath had an insertion/deletion (INDEL) sequence (≈90 kb) with seven putative genes, absent from the Nipponbare genome (Fig 1.4). Among the seven gene marker, one gene encoding a serine/threonine receptor-like cytoplasmic protein kinase was annotated by *Pup1*-K46 marker (Chin et al., 2010; 2011) and later termed as *phosphorus-starvation tolerance 1* (*PSTOL1)* (Gamuyao et al., 2012). The *PSTOL1* is upregulated under P shortage and exclusively expressed in crown root meristems at the stem base. The *PSTOL1* acts on early crown root growth by increasing root length and total root surface area, leading to a larger root system, thereby absorbing more total P concentration under the P starvation (Gamuyao et al., 2012).

To spread the uses of the *Pup1 (PSTOL1)* in rice improvement, several gene-based markers of *Pup1* locus were applied for screening germplasm and/or marker-assisted selection under P shortage (Chin et al., 2010; 2011). Among them, *Pup1-K46* (523 bp), in addition to *PSTOL1* annotation, is an informative diagnostic marker to confer P-deficient tolerance. This gene-based marker is highly conserved in the P-deficient tolerance rice cultivars, which are mainly upland ecotype or drought-tolerant cultivars (Chin et al., 2011). This *Pup1* locus is widely distributed in not only Asian rice (*O. sativa*) but also other *Oryza* species such as African rice (*O. glaberrima)* and wild rice (*O. rufipogon)* (Pariasca-Tanaka et al., 2014; Neelam et al., 2017). However, since the *Pup1*-K46 is mostly not present in lowland rice cultivars, study and application of the *Pup1*-K46 remain unclear in lowland rice cultivars.

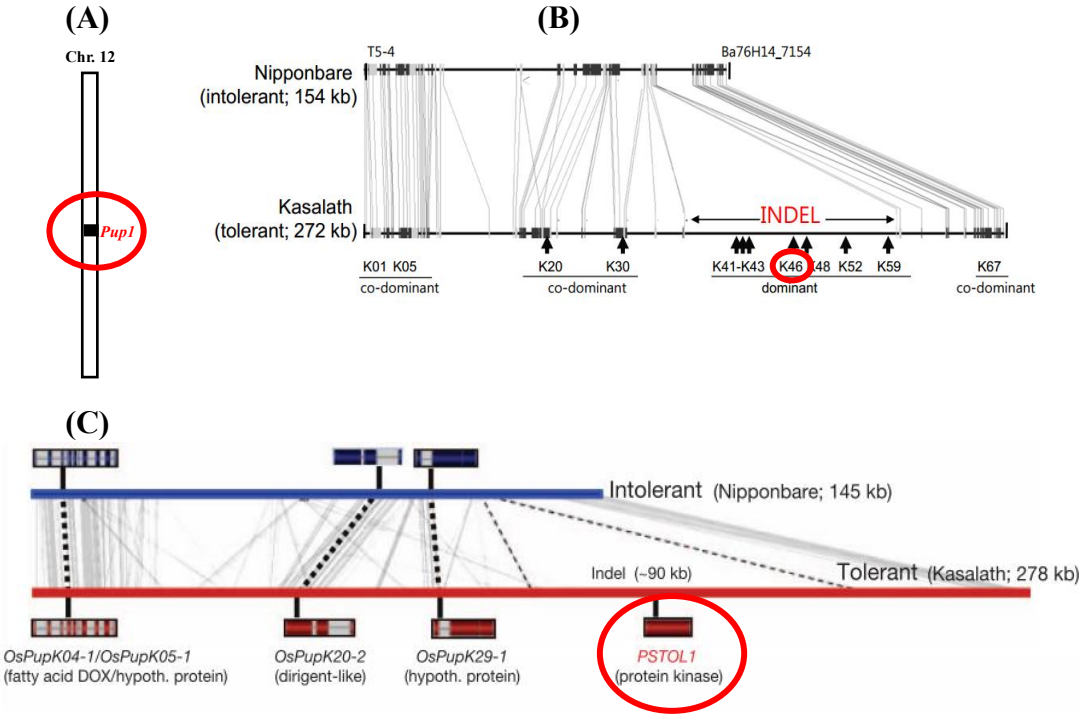


Figure 1.4 Location of the *Pup1*-K46 on rice chromosome 12. (A) The *Pup1* locus on the rice chromosome 12. (B) The dominant *Pup1* markers from K41-K59 including K46 (*Pup1*-K46) are located in the INDEL region of the P-deficient tolerance Kasalath but not existed in that of the P-deficient intolerance Nipponbare. (C) The annotation of the *Pup1*-K46 marker in the Kasalath for the *PSTOL1* gene encoding a protein kinase if comparing (B) with (C) (See details in Chin et al., 2011; Gamuyao et al., 2012).

1.3 Research purpose

The objectives of this study were:

(1) To investigate the growth performance of lowland *indica* rice cultivars mainly originated from Southern Thailand to low P availability based on the *Pup1*-K46 marker.

(2) To determine their P-deficient tolerance adaptation strategies in the selected Thai rice cultivars based on the *Pup1*-K46 marker.

CHAPTER 2
RESEARCH METHODS

To study whether the lowland rice cultivars detected with the *Pup1*-K46 are tolerant to P deficiency, our research methods contained 3 main parts: screening of the *Pup1*-K46 locus variation, test of P-deficient tolerance with rice seedlings grown in half-strength Yoshida solution (Table S2) (preliminary experiment), and test of P-deficient tolerance with rice seedlings grown in full-strength Yoshida solution (Table S3) (main experiment; Fig 2.1).

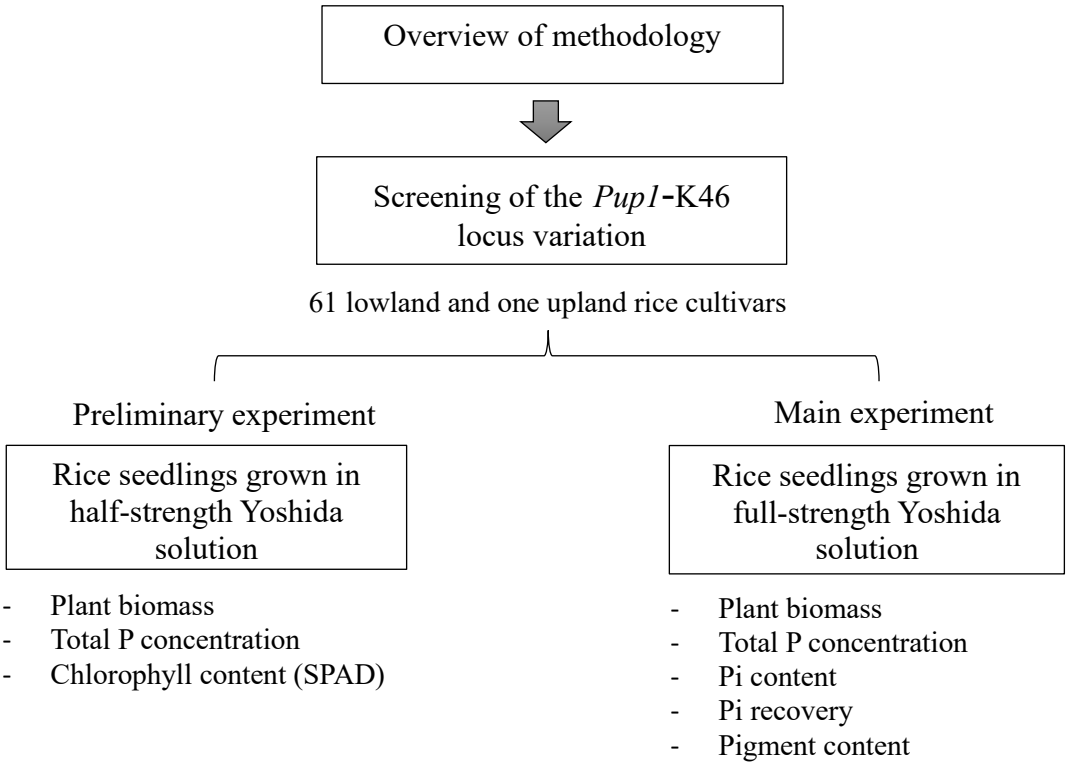


Figure 2.1 Overview of methodology in this research

2.1 Rice cultivars and seed collection

Seeds of the sixty-one lowland and one upland rice cultivars were kindly provided by Phattalung Rice Research Center, Phattalung, Thailand, and were gathered from local rice farmers from the major rice producing sites of southern Thailand such as Songkhla, Nakon Sri Thammarat and Phatthalung provinces under Plant Genetic Conservation Project under the Royal Initiative of Her Royal Highness Princess Maha Chakri Sirindhorn. Additionally, the seeds of IR64 and Dular were received from the Department of Agriculture, Ministry of Agriculture and Cooperatives, Bangkok, Thailand.

2.2 Screening of the *Pup1*-K46 locus variation

Seven-day-old seedlings of each cultivar were extracted for their genomic DNA using a protocol modified from Dellaporta et al. (1983). Twenty-five microliter PCR reaction mixture consisted of about 100 ng of the genomic DNA, 1X Vi Buffer S, 0.1 mM dNTPs mix, 0.4 µM each forward and reverse primers and 1-unit Taq DNA polymerase (Vivantis). The 523-bp *Pup1*-K46 fragment based on Chin *et al*. (2011) was determined using the *Pup1-K46* marker primers (*Pup1*-K46-F: 5′-TGAGATAGCCGTC AAGATGCT-3′ and *Pup1*-K46-R: 5′-AAGGACCACCATTCCATA GC-3′). The PCR was clarified by the *EF1α* primers as a positive control (*EF1α*-F: 5′-TTTCACTCTTG GTGTGAAGCAGAT-3′ and *EF1α*-R: 5′-GACTTCCTTCACGATTTCATCGTAA-3′) which yielded an 805-bp DNA fragment (Maksup et al., 2013). The PCR conditions were programmed as follow: initial denaturation at 95°C for 5 min, followed by 35 cycles of 3 steps PCR as denaturation at 95°C for 30 sec, annealing at 55°C for 30 sec and extension at 72°C for 1 min as well as additional final extension at 72°C for 5 min.

2.3 Plant growth conditions

According to seed germination and survival rate, 20 and 21 cultivars with and without the *Pup1*-K46 locus were selected for the preliminary experiment, and 20 cultivars with/without *Pup1*-K46 locus was selected for the main experiment, respectively (Table S1). The seeds were sowed on wet tissue papers in a petri dish in the dark. One week after seed germination, each seedling was gently transferred into 1 ml pipette tip by cutting the end of the tip for 2-3 mm to allow root growth into a pipette

tip box, which contained 500-ml Yoshida solution (Yoshida et al., 1976), one with HP and the other with LP, and then transferred to an opened greenhouse (≈12 h day/night).

To evaluate the P-deficient tolerance in the lowland rice cultivars, the seedlings were cultured in hydroponic solutions based on the concentrations of the nutrients (Fig 2.2). For the preliminary experiment, the seedlings were grown under half-strength solution, which contained 5 mg/l P for HP and 0.25 mg/l P for LP in an open greenhouse for three weeks. Nineteen to 22 seedlings (n = 19-22) of each cultivar were harvested from both treatments. The half-strength hydroponic solution was prepared as Yoshida solution as described in Table S2 (Yoshida et al., 1976).

For the main experiment, 12 seedlings (n = 12) of each cultivar were grown under HP (10 mg/l P) and LP (0.5 mg/l P) Yoshida full-strength solution in the open greenhouse for four weeks. The hydroponic solutions were changed twice a week, while the amount of solutions was daily maintained by distilled water. The full-strength hydroponic solution was prepared as Yoshida solution as described in Table S3 (Yoshida et al., 1976).

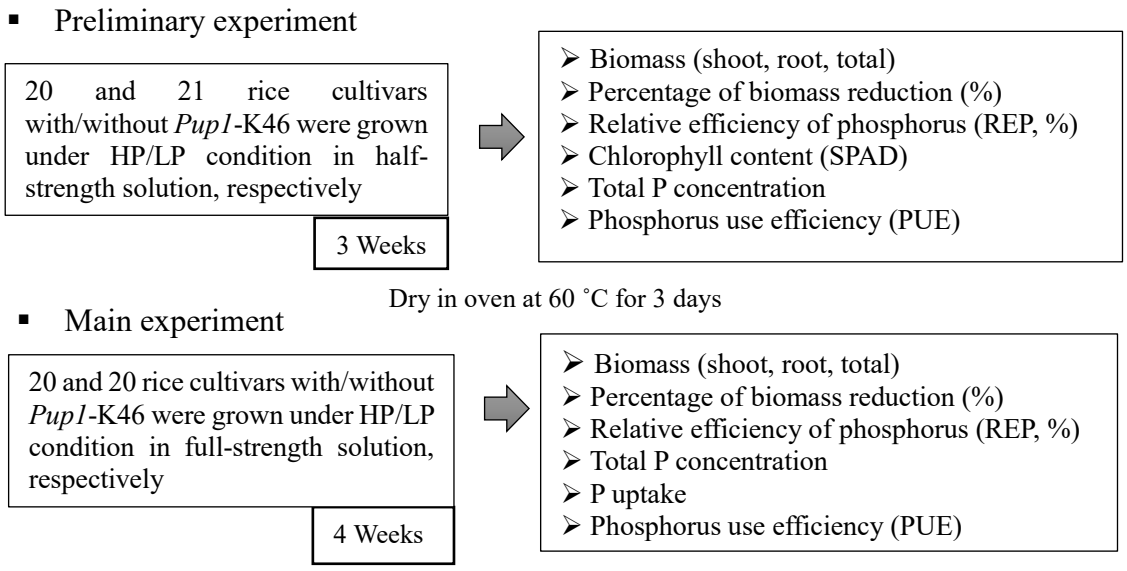


Figure 2.2 Experimental design of rice grown under P deficiency. For the preliminary experiment, the half-strength solutions with 5 mg/l P and 0.25 mg/l P are used as HP and LP, respectively; while the full-strength solutions are composed of the 10 mg/l P and 0.5 mg/l P as HP and LP in the main experiment. After 3-4 weeks, the rice plants were harvested and measured for their biomass, P concentration, Pi content and pigment content.

2.4 Plant measurements

2.4.1 Plant biomass

Three- or four-week rice seedlings were dried in an oven at 60 ˚C for three days. Shoot, root, and total biomass were individually measured. Data were presented in the percentage of dry mass reduction (Chankaew et al., 2019) and the relative efficiency of phosphorus (REP, %) (Neto et al., 2016). The dry mass reduction of the rice seedlings was calculated as the following;

$$\%\ \text{of dry mass reduction} = \frac{\mathrm{DM}_{\text{under HP}} - \mathrm{DM}_{\text{under LP}}}{\mathrm{DM}_{\text{under HP}}} \times 100$$

Where DM = average of dry mass (mg), HP = high P condition, LP = low P condition. The REP was calculated as the ratio between the plant DM under the LP and DM under the HP as the following formula;

$$\%\ \mathrm{REP} = \frac{\mathrm{DM}_{\text{under LP}}}{\mathrm{DM}_{\text{under HP}}} \times 100$$

2.4.2 Total P concentration

To analyze total P concentration in rice, dry shoot and root tissues from two plants were pooled into a sample. Three samples (3 biological replicates) were required for the analyses. Briefly, about 100 mg of the shoot and 20 mg of root were weighed with a four-digit scale and then recorded. The samples were incubated in 1 ml for root to 2 ml for shoot of 65% HNO_3 at 95ºC for an hour before adding 0.5 to 1 ml of 30% H_2O_2 for 30 minutes to complete the digestion, respectively. The solution was filtered with a Whatman paper No.1. The solution volume was then adjusted by distilled water to 10 ml. Total P concentration was measured by ICP-OES (AVO 500, Perkin Elmer).

P uptake and P use efficiency (PUE) could be calculated from the total P concentration. P uptake (mg/plant) was calculated by the average of the DM in each cultivar multiplied by P concentration as following;

P uptake (mg/plant) = DM (mg) × P concentration (mg P/Kg dry mass)

PUE was calculated as DM produced per unit P accumulated in shoot or root tissue as following (Aluwihare et al., 2016);

PUE = 1 / P concentration

2.4.3 Pi content and Pi recovery

To measure Pi accumulation in rice tissues, three P treatments which are HP, LP and P resupply (PR) were set up (Fig 2.3). Six seedlings were grown under HP and LP conditions in an open greenhouse for 4 weeks. One day before harvest, all of the seedlings were transferred to a growth-chamber under 30˚C, 70% humidity and 180 μE $m^{-2}s^{-1}$ light intensity with a 12h day and night cycle as so-called acclimatization. On the day of harvest, the hydroponic solution was renewed. For PR treatment, the seedlings from the LP solution were shifted into the HP solution for 10 hours. After 10 hours, the seedlings were harvested and frozen in liquid nitrogen for a while before storing at −80°C in a deep freezer.

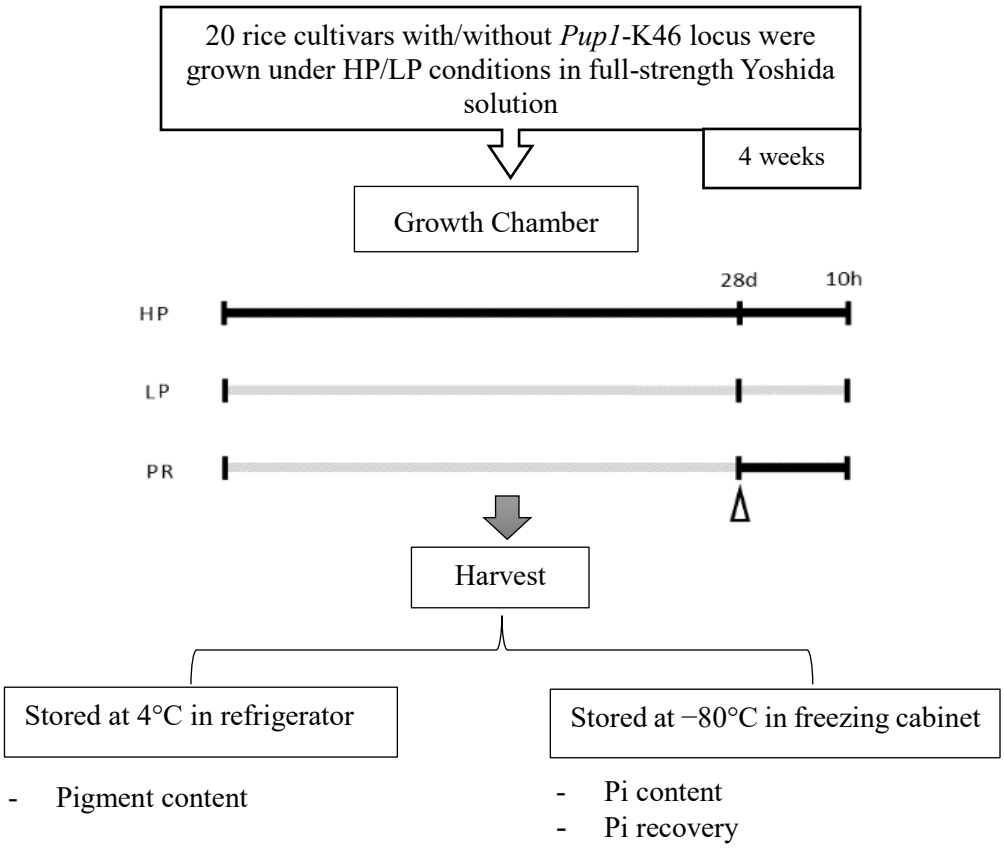


Figure 2.3 Schematic diagram of the PR experiment. Schematic lines represent the experimental designs for HP, LP, and PR treatments. The line is not drawn on a scale of day and hour. Seedlings were grown in the HP (black line) and LP (gray line) solutions for 28 days followed by PR for 10 hours. The harvested seedlings were then stored at 4°C in a refrigerator for pigment content measurement and at −80°C in freezing cabinet for Pi content and Pi recovery determination.

To measure cytoplasmic Pi contents, the second and third top fully expanded leaves were pooled into a sample. About 20 mg of leaves and 40 mg of roots were placed in 1.5 ml tubes and ground by glass pestles. The ground leaves or roots were added with 600 µl of 3% perchloric acid ($HClO_4$), then centrifuged at 11,000 rpm for 10 min and finally gently collected the 600 µl supernatant before adding 400 µl of ferrous sulfate-ammonium molybdate reagent using cuvettes as described in Hurry et al. (2000). Inorganic Pi was measured by spectrophotometer at the wavelength of 720 nm. The absorbance at 720 for each extracted tissue sample was compared to a standard curve prepared in advance with certified 0.01M KH_2PO_4 standard in the range of 0-75µl to make 1 ml solution (Table 2.1). Using the linear regression equation of the standard curve, the Pi content of each tissue sample was finally calculated and recorded (Fig 2.4).

Pi recovery (%) was calculated from the subtraction of average Pi content between PR and LP divided by the Pi content under the HP multiplied by 100;

$$\text{Pi recovery (\%)} = \frac{\text{Pi content}_{\text{ under PR}} - \text{Pi content}_{\text{ under LP}}}{\text{Pi content}_{\text{under HP}}} \times 100$$

Translocation factor was calculated from the concentration of Pi found in shoot divided by Pi found in the root of rice tissue;

$$\text{Translocation factor} = \frac{\text{Pi content found in shoot}}{\text{Pi content found in root}}$$

Table 2.1 The prepared standard curve to calculate Pi content (Hurry et al., 2000)

Pi molecules in 1 ml (nmol)	0.01 M KH_2PO_4 (µl)	3% $HClO_4$ (µl)	Assay reagent (µl)	OD_{720}
0	0	600	400	0
50	5	595	400	0.188
100	10	590	400	0.359
150	15	585	400	0.497
200	20	580	400	0.666
250	25	575	400	0.831
500	50	550	400	1.524
750	75	525	400	2.124

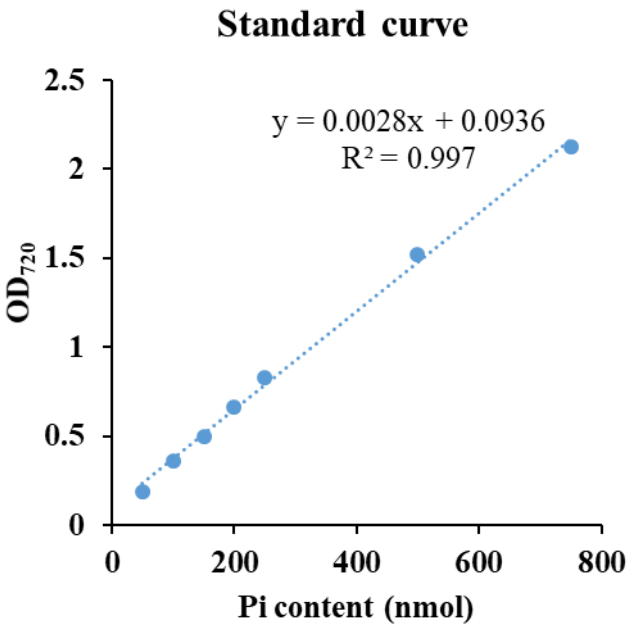


Figure 2.4 Pi content standard curve for Pi content test in rice tissue.

2.4.4 Pigment content

Pigment content was measured in the rice leaf under both half- and full-strength Yoshida solution. In the half-strength solution, the chlorophyll content was measured by chlorophyll meter by putting the device in the middle of the third leaves of the rice. Each cultivar with/without *Pup1*-K46 locus had 10 biological replicates (n = 10). In full-strength solution, the second and third top fully expanded leaves were harvested after 4 weeks and pooled into a sample. The fresh weight (5–7 mg) of middle part of the second and third leaves of each sample was recorded. The leaves were cut into small pieces and deepened in 1.5 ml tube filled with 1 ml of acetone 80 % (v/v) containing 20% (v/v) 0.2 M Tris–HCl (pH 8) and kept in the dark in the refrigerator at 4˚C (Hu et al., 2013). Each cultivar with four biological replicates (n = 4) were used for the analysis. Pigment contents were measured by Micro-plate reader (Power wavex, Biotex, USA) at Scientific Equipment Center (SEC), PSU. The absorbance was used to quantify at four different wavelengths (480, 510, 645 and 663 nm) which are described by the formula below (Kaewubon et al., 2015);

$$\text{Total Chl} = \frac{[20.2(A_{645}) + 8.02\,(A_{663})] \times V}{1000 \times FW}$$

$$\text{Chl a} = \frac{[12.7(A_{663}) + 2.63\,(A_{645})] \times V}{1000 \times FW}$$

$$\text{Chl b} = \frac{[22.9(A_{645}) + 4.68\,(A_{663})] \times V}{1000 \times FW}$$

$$\text{Carotenoid} = \frac{[7.6(A_{480}) - 2.63(A_{510})] \times V}{1000 \times FW}$$

Where: A_{480}, A_{645}, A_{510}, A_{663} are the absorbance values at 480, 645, 510 and 663 nm, respectively.

V(ml): volume of extracted solution

FW(g): fresh weight of leaves

2.5 Statistical analysis

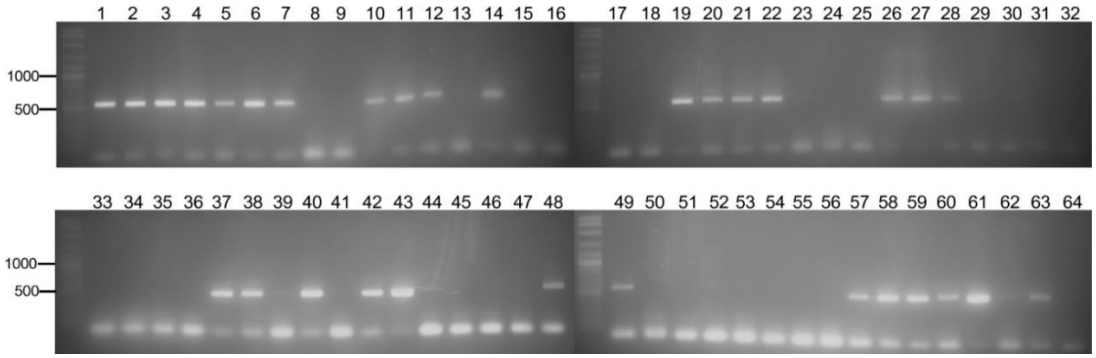
All data were statistically analyzed using Excel 2016 (Source: www.microsoft.com) and R studio 1.1.456 (Source: www.rstudio.com) software. Student's t-test was done in the Excel to compare means of dry mass reduction, REP and overall PUE between the *Pup1*-K46 positive and negative cultivars in the preliminary experiment. In the main experiment, means of dry mass reduction, REP, total P concentration, P uptake, PUE, Pi content, translocation factor, and Pi recovery efficiency were also analyzed by the t-test.

One-way ANOVA followed by LSD test was performed in the R to compare means of dry weight, total P concentration, PUE and SPAD values from each single cultivar in the preliminary experiment, as well as pigment contents in the main experiment. Correlations between P uptake and PUE and between Pi content and pigment contents of the *Pup1*-K46 positive and negative cultivars in the main experiment were also calculated by the R studio and constructed under the Excel.

CHAPTER 3
RESULTS

3.1 Investigation of *Pup1*-K46 in 61 lowland rice cultivars

To determine if the *Pup1*-K46 locus in rice cultivars is consistent with the P-deficient tolerance, we selected 61 lowland rice cultivars from southern Thailand and only one upland rice cultivar named Dokpayom. We used Dular and IR64 as positive and negative controls for genotyping analysis, respectively. After running PCR amplification using the *Pup1*-K46 primers, Dular and IR64 were detected and not detected for *Pup1*-K46 as expected, respectively (Fig 3.1). Among the 61 cultivars, only 31 contained the *Pup1*-K46 region (Fig 3.1). The PCR results of *Pup1*-K46 were all confirmed by *EF1α* marker as a positive control (Fig 3.2).



Cultivars with *Pup1*-K46		Cultivars without *Pup1*-K46	
(1) Khao Leuang	(27) Khao Paen	(8) Look Non	(35) Glib Mek
(2) Kaen Chan	(28) Look Pueng	(9) Chuk Tium Dang	(36) Klah Nak
(3) Chaw Pli Khao	(37) Meuang Sai	(13) Look Khuey	(39) Sae Mah
(4) Mae Mai	(38) Maw Arun	(15) Look Taw	(41) Tia Malay Daeng
(5) Hal Ham Doo Lila	(40) Sahn Suay	(16) Chana	(44) Chaing
(6) Kilo	(42) Rice Berry	(17) Yah Go Ba	(45) Khem Thawng
(7) Chaw La Mai	(43) Leb Nok	(18) Man Tom	(46) Look Plah
(10) Hua Nadam	(48) Khao Dam	(23) Dawk Payorm	(47) Khao Mae
(11) Buang Khao	(49) Sang Yod	(24) Koo Muang leuang	(50) Malay
(12) Eaw Mod Daeng	(57) Hawm Bai-Tuey	(25) Khom	(51) Ai-chaing
(14) Ma Jah Nu	(58) Hawm Tammasat	(29) Rai Sai	(52) RD41
(19) Nahng Long	(59) RD15	(30) Poo Yoo	(53) Hawm Rachinee
(20) Lam Hak	(60) Pathumthani 1	(31) Khao Rak	(54) Mlea Hawm
(21) Ra den	(61) KDML105	(32) Nahng Loi	(55) Khao Kem Thawng
(22) Look Dam	(62) RD61	(33) Puang Wai	(56) Hom Nil
(26) Koo Ning	(63) Dular	(34) Gan Tang	(64) IR64

Figure 3.1 Genotyping analysis of the *Pup1*-K46 marker in Thai lowland rice cultivars. Dular (63) and IR64 (64) were used as positive and negative controls. Major bands of DNA ladder at 500 and 1,000 bp were labeled on the left and center of the gels.

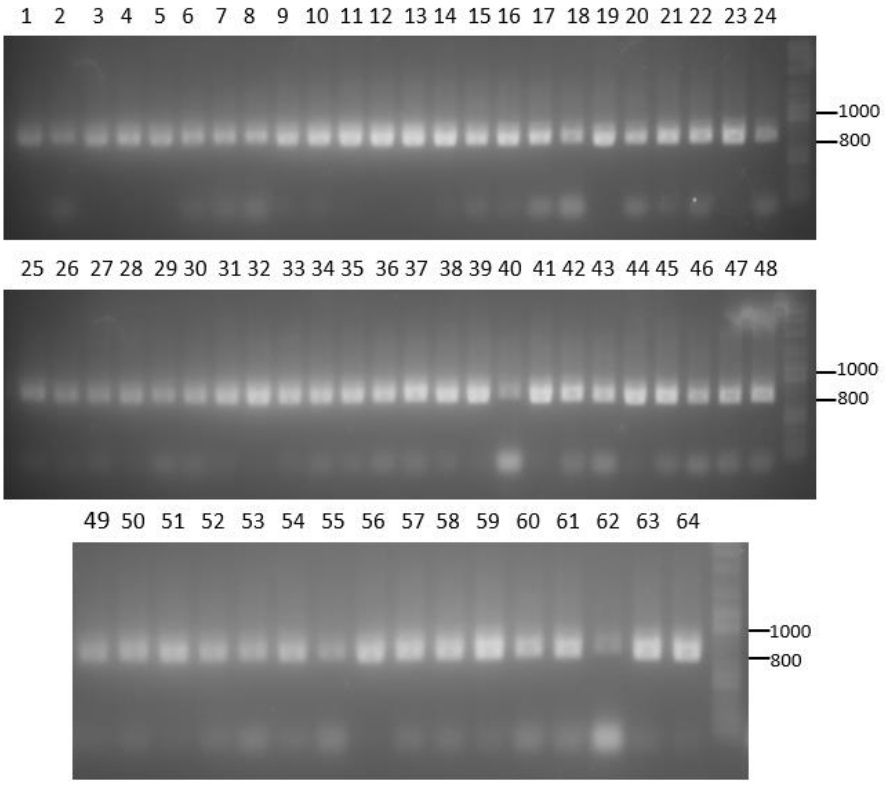


Figure 3.2 Genotyping analysis of *EF1α* marker in Thai lowland rice cultivars. Dular (63) and IR64 (64) were used as positive and negative controls. The major bands of DNA ladder at 800 and 1,000 bp were labeled on the right of the gels.

3.2 Preliminary determination of growth responses of the *Pup1*-K46 positive and *Pup1*-K46 negative cultivars to low P availability in half-strength solution

3.2.1 Effects of P deficiency on the rice biomass

Twenty and 21 rice cultivars with and without *Pup1*-K46 locus, respectively, were selected to confirm the role of *Pup1*-K46 in rice growth performance under the HP and LP conditions in half-strength solution (Table S1). One-week-old seedlings were treated under the HP and LP solutions and placed in a greenhouse for 21 days (Fig 3.3A). We considered shoot, root and total dry mass as parameters to confirm rice growth under the two different P treatments. Result showed that compared with the HP condition (Fig 3.3B), the LP condition (Fig 3.3C) seemingly reduced shoot and root biomass in all rice cultivars (Table S4).

Figure 3.3 Rice seedlings grown under the half-strength solution. (A) The seedlings were placed into pipette tip boxes which contained 5 mg/l and 0.25 mg/l P for the HP (left box) and LP (right box) conditions, respectively. Compared to (B) the HP condition, (C) the LP condition reduced the vegetative growth of the seedlings.

Responding to the genotyping analysis, all the cultivars with/without *Pup1*-K46 locus were classified into *Pup1*-K46 positive (*Pup1*-K46^{+}) and negative (*Pup1*-K46^{-}) cultivars, respectively. The *Pup1*-K46^{+} and *Pup1*-K46^{-} cultivars were verified by growing under the LP solution and recording their relative percentage of biomass reduction. The results indicated that the *Pup1*-K46^{+} cultivars had significantly lower percentage of shoot dry mass reduction than *Pup1*-K46^{-} cultivars ($P = 0.040$; Fig 3.4A), whereas there were no significant differences in the root (Fig 3.4B) and total dry mass reduction (Fig 3.4C) of the two distinct *Pup1*-K46 cultivars. It can alternatively be presented by REP (%). Due to P-deficient tolerance, the *Pup1*-K46^{+} cultivars had higher shoot REP than the *Pup1*-K46^{-} cultivars ($P = 0.040$; Fig 3.5A), while the root (Fig 3.5B) and total REP (Fig 3.5C) between the two groups were not significantly different.

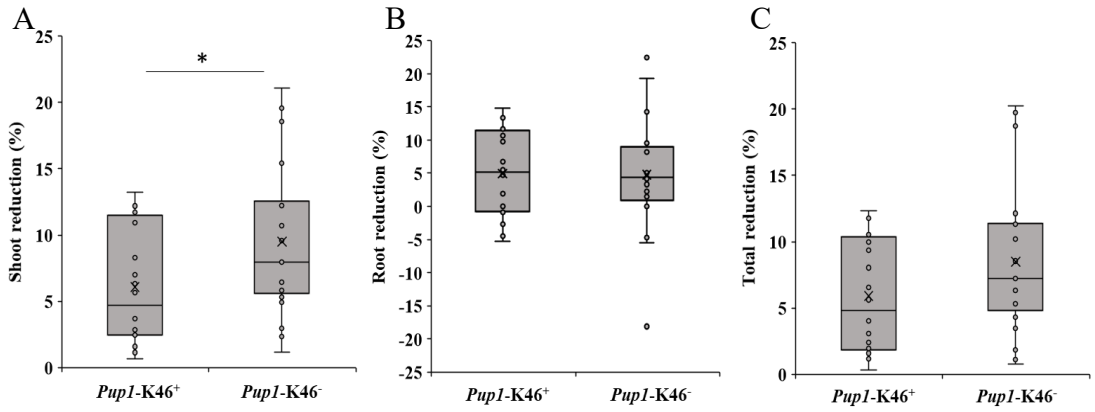


Figure 3.4 Percentages of dry mass reduction under half-strength solution. (A) Shoot, (B) root, and (C) total dry mass reduction of the *Pup1*-K46^{+} and *Pup1*-K46^{-} cultivars. The horizontal lines in the boxes are median values of the 20 and 21 cultivars for *Pup1*-K46^{+} and *Pup1*-K46^{-} cultivars, respectively (n = 20 and 21, respectively). Error bars represent standard deviation (SD). Statistical analysis was conducted with Student's t-test. *, **, and *** indicate significant differences at $P \leq 0.05$, 0.01, and 0.001, respectively.

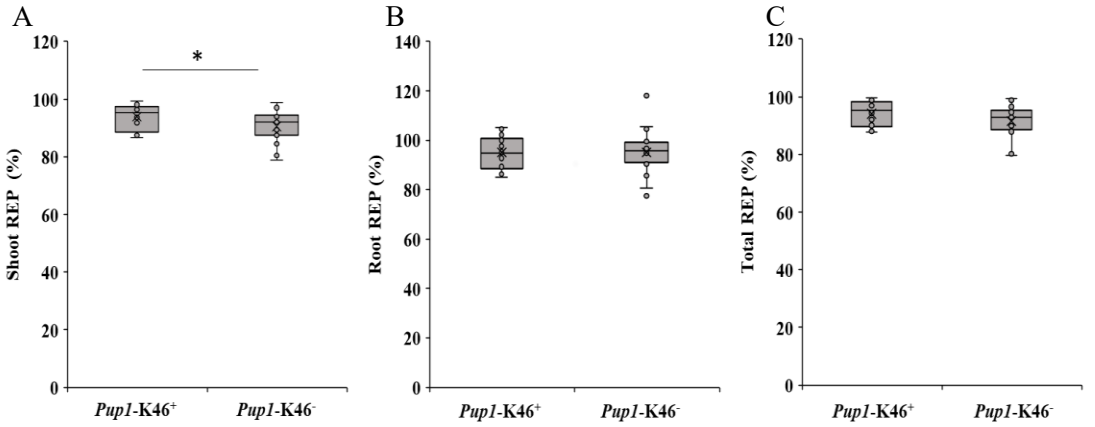


Figure 3.5 Relative efficiency of P use (REP) under half-strength solution. (A) Shoot, (B) root, and (C) total REP of the *Pup1*-K46^{+} and *Pup1*-K46^{-} cultivars. The box plots show the distribution of the percentage of REP. The horizontal lines inside the boxes indicate the median values of the 20 and 21 cultivars for *Pup1*-K46^{+} and *Pup1*-K46^{-} cultivars, respectively (n = 20 and 21, respectively). Error bars represent SD. Statistical analysis was conducted with Student's t-test. *, **, and *** indicate significant differences at $P \leq 0.05$, 0.01, and 0.001, respectively.

3.2.2 Determination of P-deficient tolerance by P accumulation and PUE

To confirm whether the P treatment modifies P accumulation in rice, three rice cultivars with/without the *Pup1*-K46 locus were selected to measure total P concentration in shoot and root tissues. Three cultivars symbolizing *Pup1*-K46^{+} are Buang Khao (BK), Hom Tammasat (TU01) and Pathumthani 1 (PT1), and *Pup1*-K46^{-} are Gan Tang (KT), RD61 (RD61) and Malay (ML). Although dry weights within shoot and root were similar under HP and LP conditions (Fig 3.6A), the LP condition significantly reduced total P concentration in shoot and root tissues (Fig 3.6B). The P concentration in the shoots under the HP condition was higher than the roots, but the P concentrations in shoot and root were not different under the LP condition (Fig 3.6B). On the other hand, the LP condition significantly increased the PUE in shoot and root compared with the HP condition. The seedlings grown under the LP condition had significantly higher overall shoot PUE in the *Pup1*-K46^{+} cultivars compared with the *Pup1*-K46^{-} cultivars (Fig 3.6C).

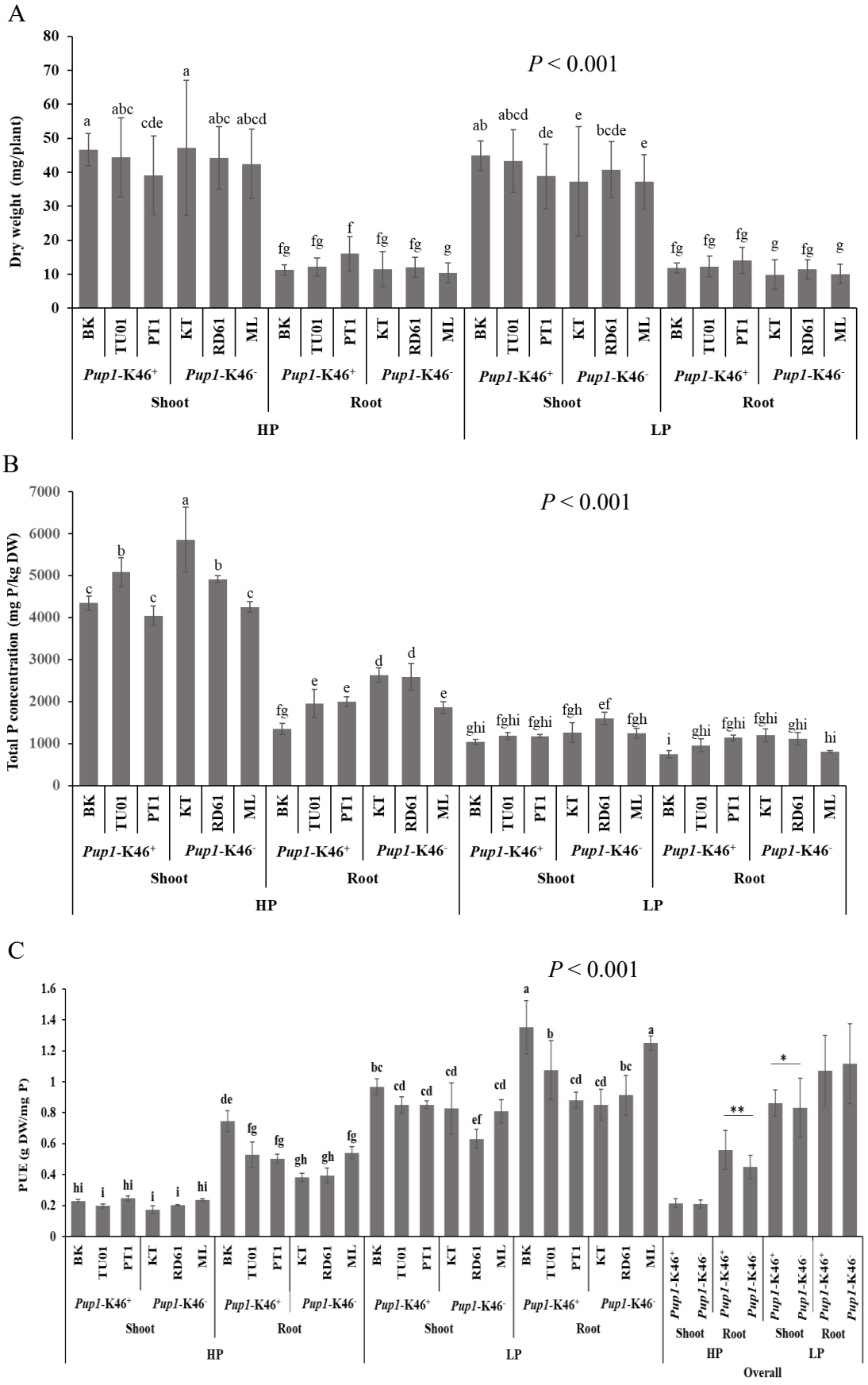

A
Dry weight (mg/plant)
P < 0.001
B
Total P concentration (mg P/kg DW)
P < 0.001
C
PUE (g DW/mg P)
P < 0.001
BK
TU01
PT1
KT
RD61
ML
Pup1-K46+
Pup1-K46-
Shoot
Root
HP
LP
Overall

Figure 3.6 Determination of dry weight, total P concentration and PUE under half-strength solution. (A) Dry weight (n = 19-22), (B) total P concentration (n = 3) and (C) PUE (n = 3) in shoot and root tissues of the *Pup1*-K46^{+} and *Pup1*-K46^{-} cultivars under the HP and LP conditions. Error bars represent SD. Different letters show significant differences at 5% level (ANOVA, LSD test; $P \leq 0.05$). Asterisks show significant differences between the *Pup1*-K46^{+} and *Pup1*-K46^{-} cultivars (Student's t-test). *, **, and *** indicate significant differences at $P \leq 0.05$, 0.01, and 0.001, respectively.

3.2.3 Effect of P deficiency on chlorophyll contents in rice leaves by chlorophyll meter

To verify whether the LP condition reduces chlorophyll contents in leaves of the rice seedlings, the alternation of leaf color was estimated by chlorophyll meter. The result showed that there were significant differences in the SPAD values, which reflect chlorophyll content, observed in the rice leaves, treated by the HP and LP conditions ($P < 0.001$). The LP condition increased the SPAD values based on the HP condition both in the *Pup1*-K46^{+} and *Pup1*-K46^{-} cultivars (Fig 3.7; Table S5).

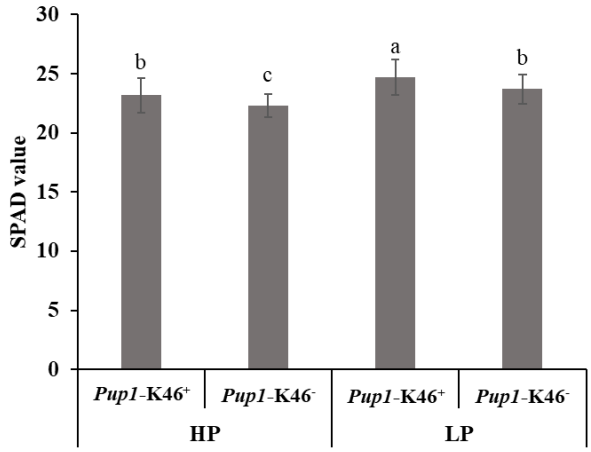


Figure 3.7 SPAD values in the rice leaves under half-strength solution. The values were measured by chlorophyll meter in the *Pup1*-K46^{+} and *Pup1*-K46^{-} cultivars under the LP and HP conditions. Data correspond to the average of the values from 20-21 cultivars (n = 20-21). Error bars represent SD. Different letters show significant differences of the SPAD values (ANOVA, LSD test; $P \leq 0.05$).

3.3 Determination of growth responses of the *Pup1*-K46 positive and *Pup1*-K46 negative cultivars to low P availability in full-strength solution

3.3.1 Effects of P deficiency on the rice biomass

Previously, we tested rice growth performances in half-strength Yoshida solution and found that LP condition reduced rice growth. Moreover, the *Pup1*-K46 positive groups were more tolerant to P deficiency, compared with the *Pup1*-K46 negative groups. However, the half-strength solution might contain insufficient nutrients especially nitrogen, which might affect the evaluation of P-deficient tolerance in the seedlings. The following experiment was conducted to confirm the rice growth in full-strength Yoshida solution. Twenty cultivars with/without *Pup1*-K46 were selected to confirm the role of the *Pup1*-K46 on rice growth performance under the HP and LP conditions in full-strength Yoshida solution (Table S1). The seedlings were placed in an open greenhouse for four weeks (Fig 3.8A). After 28 days, the lengths of the seedlings were measured with a ruler (Fig 3.8B). The result showed that the LP condition reduced shoot biomass in all rice cultivars and root biomass in some cultivars compared to the HP condition (Fig 3.8C; Table S6). Under the LP condition, root to shoot ratio was significantly increased in almost all of the cultivars (Table S6).

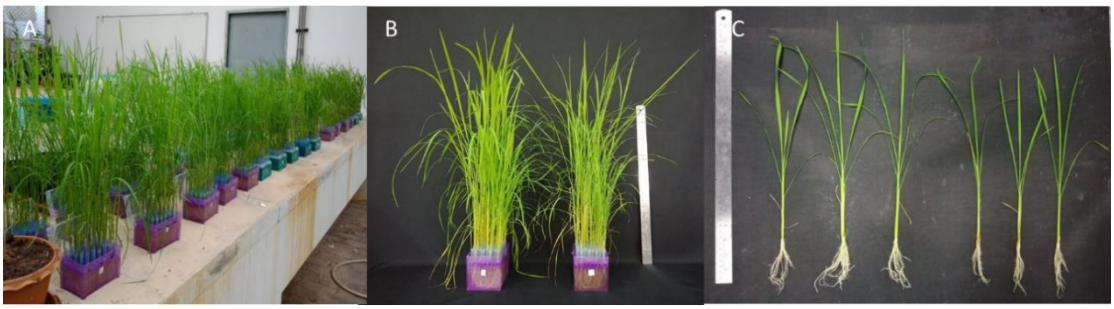


Figure 3.8 Rice seedlings grown under full-strength solution. (A) The seedlings were placed into pipette boxes containing 10 mg/l (HP) and 0.5 mg/l P (LP) in an open greenhouse. (B) The seedlings were cultured under the HP (left box) and LP (right box) conditions. (C) Compared to the HP condition (left), the LP condition reduced the vegetative growth of the seedlings (right).

The *Pup1*-K46^{+} and *Pup1*-K46^{-} cultivars under the LP condition were clarified based on the shoot, root, and total biomass reduction. The hydroponic system showed that the percentage of shoot dry mass reduction in the *Pup1*-K46^{+} cultivars was significantly lower than in the *Pup1*-K46^{-} cultivars (P = 0.027; Fig 3.9A), whereas the root (Fig 3.9B) and total dry mass reductions (Fig 3.9C) were not significantly different. The *Pup1*-K46^{+} cultivars were significantly greater in REP (%) than the *Pup1*-K46^{-} cultivars as they produced the greater biomass in the shoot (P = 0.027; Fig 3.10A). The REPs in the root (Fig 3.10B**)** and total biomass (Fig 3.10C) were not significantly different.

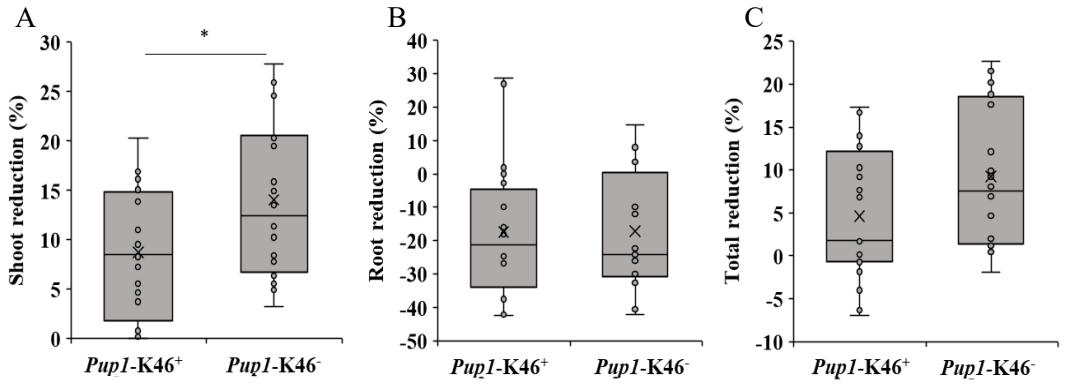


Figure 3.9 The percentage of dry mass reduction under full-strength solution. (A) Shoot, (B) root, and (C) total biomass reduction of *Pup1*-K46^{+} and *Pup1*-K46^{-} cultivars. The horizontal lines inside the boxes are the median values of 20 cultivars (n = 20). Error bars represent SD. Statistical analysis was conducted with Student's t-test. *, **, and *** indicate significant differences at $P \leq 0.05$, 0.01, and 0.001, respectively.

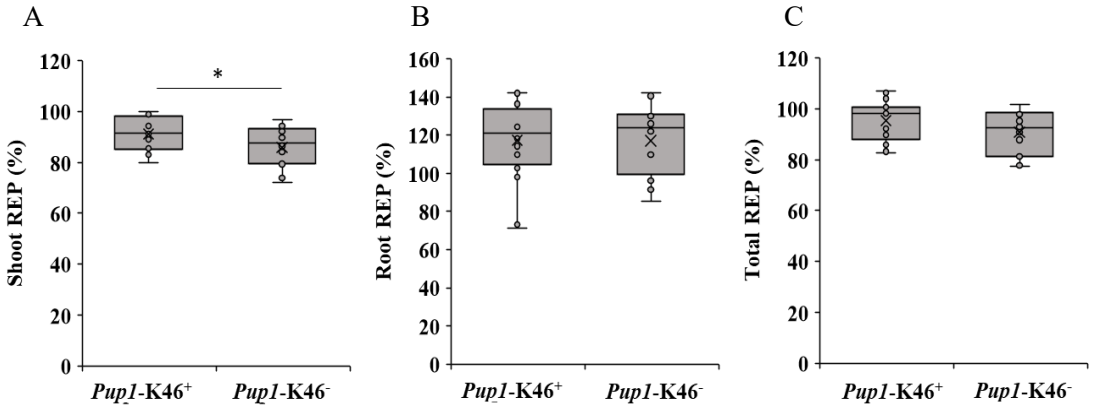


Figure 3.10 Relative efficiency of P use (REP) under full-strength solution. (A) Shoot, (B) root, and (C) total REP of the *Pup1*-K46$^+$ and *Pup1*-K46$^-$ cultivars. The box plots show the distribution of the percentage of REP. The horizontal lines inside the boxes indicate the median values of the 20 cultivars (n = 20). Error bars represent SD. Statistical analysis was conducted with Student's t-test. *, **, and *** indicate significant differences at $P \leq 0.05$, 0.01, and 0.001, respectively.

3.3.2 Total P concentration under P deficiency

To test our hypothesis whether the *Pup1*-K46$^+$ cultivars deposit higher total P concentrations especially under the LP condition, compared with the *Pup1*-K46$^-$ cultivars, all the cultivars with/without *Pup1*-K46 were grown under the LP and HP solutions. The experiment showed that the LP condition significantly reduced shoot and root total P concentrations in the *Pup1*-K46$^+$ and *Pup1*-K46$^-$ rice cultivars (Table S7). Both HP and LP conditions ($P = 0.005$ and 0.002, respectively) had significantly higher total P concentration in root in the *Pup1*-K46$^+$ cultivars compared with *Pup1*-K46$^-$ cultivars, but there were no differences in the shoot (Fig 3.11A).

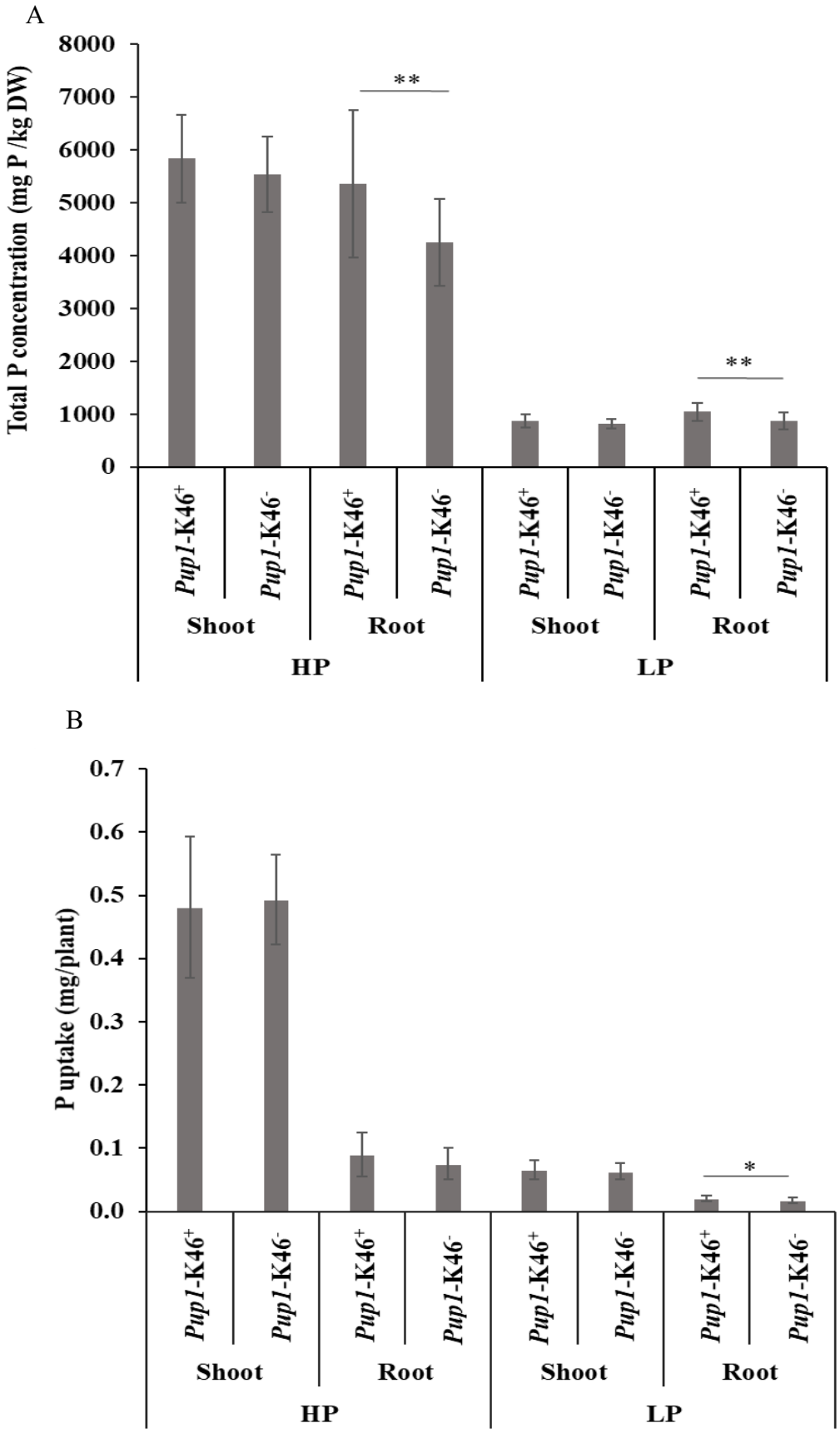
3.3.3 P uptake under P deficiency

P uptake is referred to the plant ability to store an amount of P from the solution. To determine whether the *Pup1*-K46$^+$ cultivars are more capable of taking up P under the LP environment than the *Pup1*-K46$^-$ cultivars, P uptake was calculated from dry mass multiplied by total P concentration in shoot and root. The results showed that the LP condition significantly reduced P uptake in shoot and root of the *Pup1*-K46$^+$ and *Pup1*-K46$^-$ cultivars (Table S8). Under the LP condition, the *Pup1*-K46$^+$ cultivars

highly accumulated a greater amount of P in their roots than the *Pup1*-K46^{-} cultivars (P = 0.05), but there were no differences in the shoot. Under the HP condition, there were no differences of the P uptake in the shoot and root tissues in the *Pup1*-K46^{+} and *Pup1*-K46^{-} cultivars (Fig 3.11B).

3.3.4 PUE under P deficiency

PUE is referred to the amount of biomass produced per unit P or the inverse of tissue P concentration. To apply whether PUE is a good indicator of P-deficient tolerance in rice cultivars, the PUE was calculated by the ratio of 1 to the total P concentration. The result showed that the LP condition significantly raised the PUE in shoot and root of the *Pup1*-K46^{+} and *Pup1*-K46^{-} cultivars (Table S9). The LP condition showed higher PUE in root of the *Pup1*-K46^{-} cultivars compared with the *Pup1*-K46^{+} cultivars (P = 0.001), but there were no differences in the shoot. The HP condition also showed higher PUE in root of the *Pup1*-K46^{-} cultivars compared with the *Pup1*-K46^{+} cultivars (P = 0.012), but there were also no differences in the shoot (Fig 3.11C). Besides, there were no correlations between PUE and biomass of shoot and root under the HP and LP conditions in the *Pup1*-K46^{+} cultivars, while there were significant negative correlations between PUE and biomass of shoot and root of the *Pup1*-K46^{-} cultivars in the HP condition, but not in the LP condition (Fig S1).


A
Total P concentration (mg P /kg DW)
8000
7000
6000
5000
4000
3000
2000
1000
0
**
**
Pup1-K46+
Pup1-K46-
Pup1-K46+
Pup1-K46-
Pup1-K46+
Pup1-K46-
Pup1-K46+
Pup1-K46-
Shoot
Root
Shoot
Root
HP
LP
B
P uptake (mg/plant)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
*
Pup1-K46+
Pup1-K46-
Pup1-K46+
Pup1-K46-
Pup1-K46+
Pup1-K46-
Pup1-K46+
Pup1-K46-
Shoot
Root
Shoot
Root
HP
LP

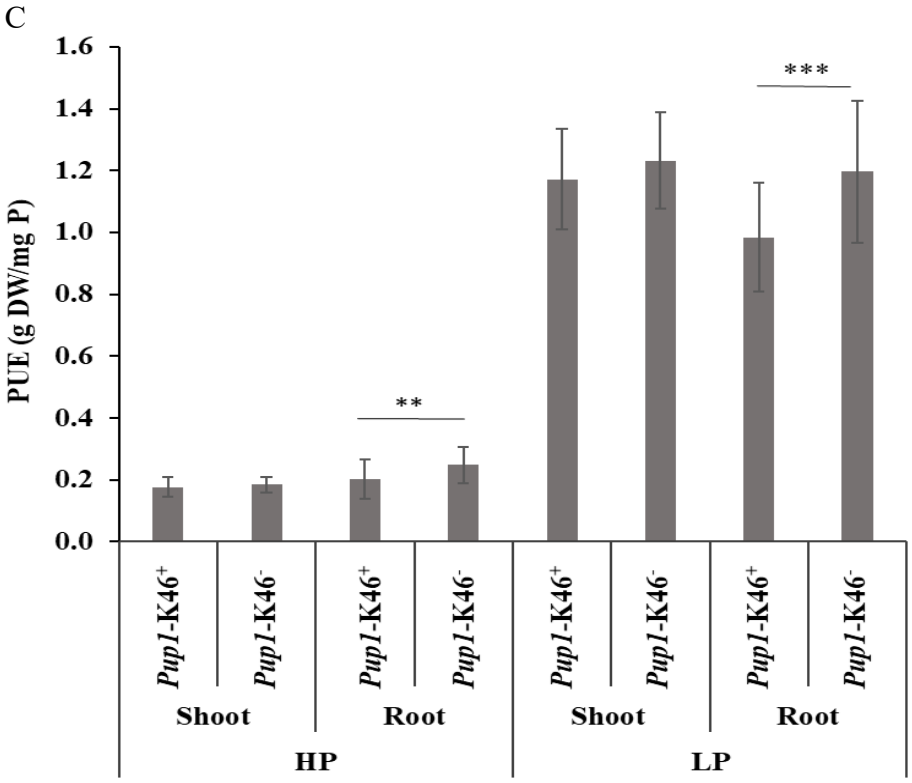


Figure 3.11 Determination of total P concentration, P uptake and PUE under full-strength solution. (A) Total P concentration, (B) P uptake and (C) PUE in shoot and root of the *Pup1*-K46^{+} and *Pup1*-K46^{-} cultivars under HP and LP condition. Data correspond to the mean of twenty biological replicates (n = 20). Error bars represent SD. Statistical analysis was conducted with Student's t-test. *, **, and *** indicate significant differences at $P \leq 0.05$, 0.01, and 0.001, respectively.

3.3.5 Relationship between P uptake and PUE under P deficiency

To investigate the relationship between P uptake and PUE of the *Pup1*-K46^{+} and *Pup1*-K46^{-} cultivars under the LP condition, the correlations between the P uptake and PUE in the shoot and root under the HP and LP conditions were statistically analyzed. The results showed that there were significant negative correlations between the P uptake and PUE in the shoot and root observed in the *Pup1*-K46^{+} and *Pup1*-K46^{-} cultivars, except shoot of the *Pup1*-K46^{-} cultivars under HP condition (Fig 3.12). P uptake and PUE in the shoot of *Pup1*-K46^{+} cultivars were significantly correlated under the HP condition (Fig 3.12A). Under the HP condition, there were also significant negative correlations between the P uptake and PUE in the root of the *Pup1*-K46^{+} and *Pup1*-K46^{-} cultivars (Fig 3.12B). Under the LP condition, there were significant negative correlations between P uptake and PUE in the shoots (Fig 3.12C) and roots (Fig 3.12D) of the *Pup1*-K46^{+} and *Pup1*-K46^{-} cultivars.

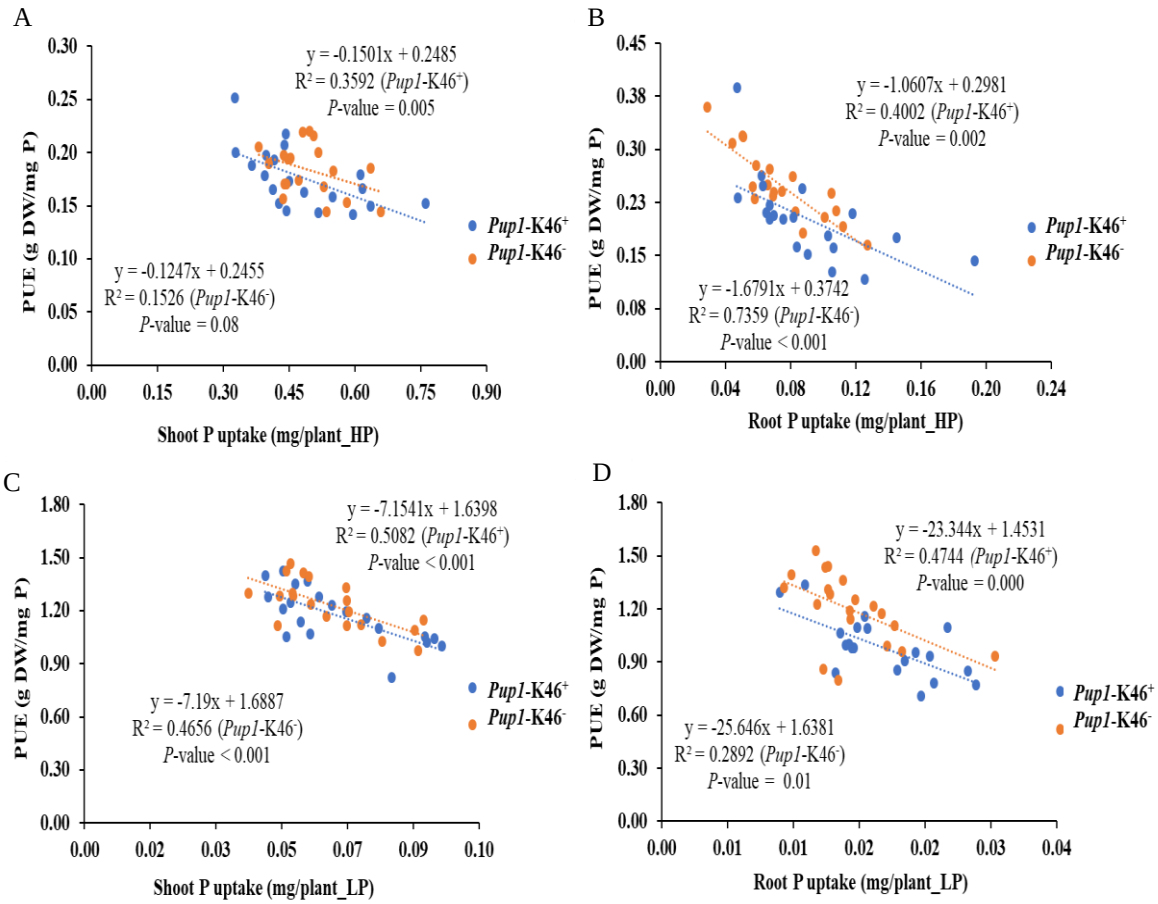


Figure 3.12 Correlations between P uptake and PUE in shoot and root of the *Pup1*-K46^{+} and *Pup1*-K46^{-} cultivars under the HP and LP conditions. Shown are the correlations between P uptake and PUE in (A) shoot and (B) root of the *Pup1*-K46^{+} and *Pup1*-K46^{-} cultivars under the HP condition, and between P uptake and PUE in (B) shoot and (C) root of the *Pup1*-K46^{+} and *Pup1*-K46^{-} cultivars under LP condition. The correlation was calculated by R software with LSD, (*P*-value $\leq$ 0.05).

3.3.6 Pi content under P deficiency

To confirm whether the *Pup1*-K46^{+} cultivars accumulate higher Pi contents than *Pup1*-K46^{-} cultivars especially under the LP condition. The seedlings were grown under the HP and LP treatments for four weeks. The results showed that the LP condition reduced Pi content in shoot and root of the *Pup1*-K46^{+} and *Pup1*-K46^{-} cultivars compared to the HP condition (Fig 3.13A). Under the LP condition, the *Pup1*-K46^{+} cultivars had significantly higher Pi content in shoot and root than the *Pup1*-K46^{-} cultivars ($P < 0.001$ and $P = 0.003$, respectively). For PR treatment, the seedlings were treated under the LP treatment for four weeks and suddenly exposed to the HP solution for ten hours. The result showed that within the short period of time, Pi content increased under the PR, but there were no differences in the shoot and root of the *Pup1*-K46^{+} and *Pup1*-K46^{-} cultivars (Fig 3.13A; Table S10). Nevertheless, the Pi reduction of rice compared when grown in HP and LP conditions of the *Pup1*-K46^{+} cultivars was significantly lower than the *Pup1*-K46^{-} cultivars in both shoot and root (Fig S2).

Translocation factor is the plant's potential to absorb Pi from soil/solution and transfer it from root to shoot. The Pi translocation factor was calculated as the ratio of Pi content found in the shoot with that Pi content found in the root of rice tissue. The LP treatment reduced the translocation factor in the *Pup1*-K46^{+} and *Pup1*-K46^{-} cultivars. Under the LP condition, there were no differences in the translocation factor between *Pup1*-K46^{+} and *Pup1*-K46^{-} cultivars. Under the PR treatment, although no differences in translocation factor were found, the *Pup1*-K46^{+} cultivars seem to have higher translocation factors than the *Pup1*-K46^{-} cultivars after exposing to the HP solution for ten hours (Fig 3.13B).

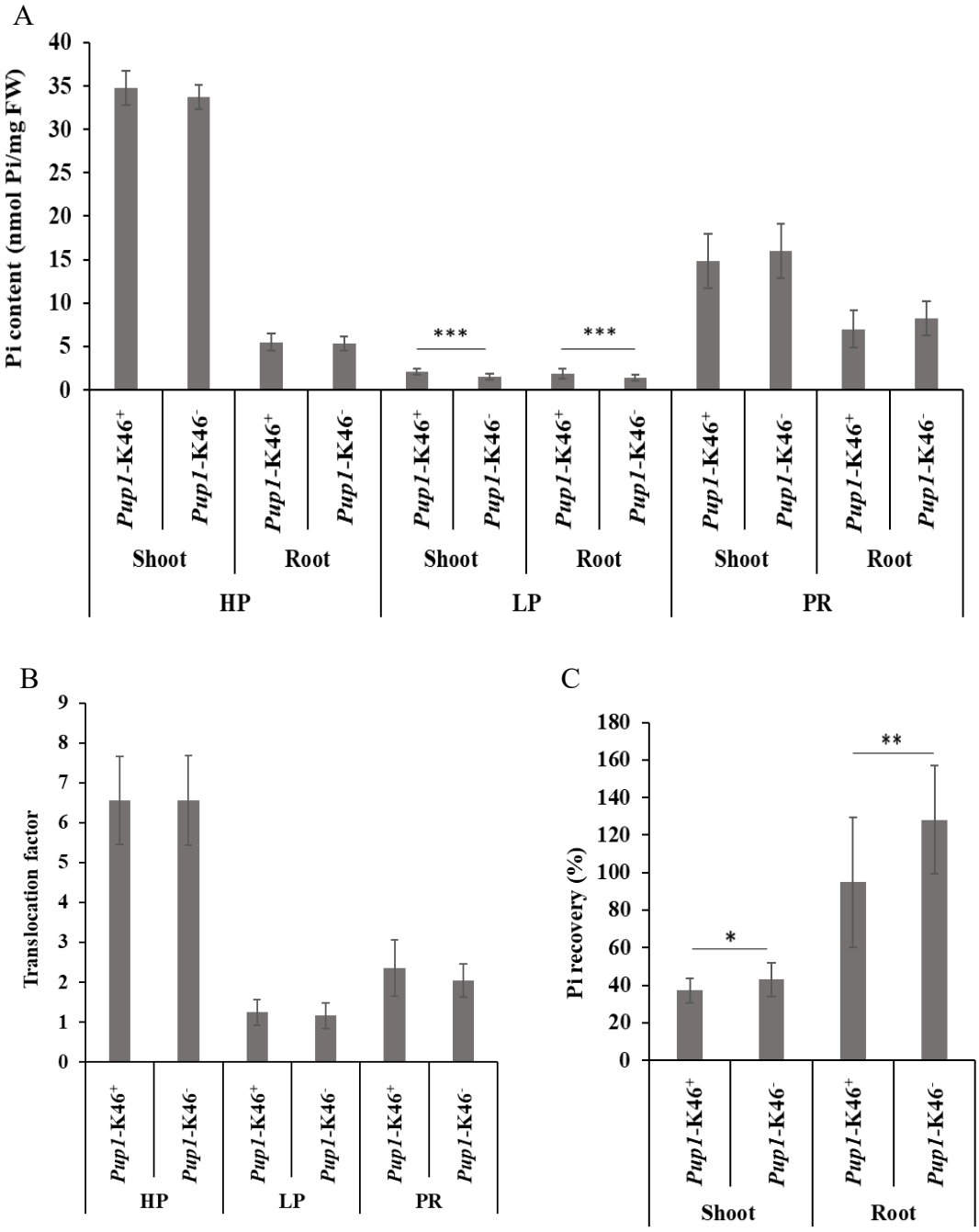


Figure 3.13 Determination of Pi content, translocation factor and Pi recovery efficiency under full-strength solution. (A) Pi content, (B) translocation factor and (C) Pi recovery efficiency of the shoot and root Pi content under the three different P regimes between the *Pup1*-K46^{+} and *Pup1*-K46^{-} cultivars. Data correspond to the mean of twenty biological replicates (n = 20). Error bars represent SD. Statistical analysis was conducted with Student's t-test. *, **, and *** indicate significant differences at $P \leq$ 0.05, 0.01, and 0.001, respectively.

3.3.7 Pi recovery efficiency after P deficiency

To check whether the *Pup1*-K46$^+$ cultivars absorb Pi faster than the *Pup1*-K46$^-$ cultivars after suddenly supplying Pi for 10 hours after 4-week LP treatment, percentage of P recovery was calculated from the subtraction of Pi content between the PR and LP conditions divided by Pi content under the HP condition multiplied by 100, representing the efficiency of Pi maintenance within 10 hours. The experiment showed that in the shoot, only 37 and 42% of Pi content were recovered in the *Pup1*-K46$^+$ and *Pup1*-K46$^-$ cultivars, respectively ($P = 0.025$). In the root, we found the over-accumulation of Pi content to be approximately 128% in the *Pup1*-K46$^-$ cultivars compared with 94% in *Pup1*-K46$^+$ cultivars ($P = 0.002$). The experiment also showed that within 10 hours, the *Pup1*-K46$^+$ cultivars recovered their Pi content slower than the *Pup1*-K46$^-$ cultivars (Fig 3.13C).

3.3.8 Pigment content changes due to P deficiency

We have already measured the chlorophyll content in rice leaves using the chlorophyll meter in the preliminary experiment and the result showed that the LP condition significantly increased the leaf greenness, which reflects the chlorophyll content indirectly, in the *Pup1*-K46$^+$ and *Pup1*-K46$^-$ cultivars. To confirm the chlorophyll contents obtained from the chlorophyll meter, the pigment content in the leaves of each cultivar under the P deficiency in the main experiment using full-strength solution were quantified. The experiments showed that all the pigment contents in leaves between the *Pup1*-K46$^+$ and *Pup1*-K46$^-$ cultivars were significantly different under the LP condition, but there were no significant differences under the HP condition (Fig 3.14). The accumulation of chlorophyll a (Fig 3.14B) and carotenoid (Fig 3.14D) under the LP condition was significantly higher in the *Pup1*-K46$^+$ cultivars, whereas it was unchanged in the *Pup1*-K46$^-$ cultivars compared with HP condition ($P < 0.05$ for chl a and $P < 0.05$ for carotenoid). Under HP condition, total chlorophyll content (Fig 3.14A) and chlorophyll b (Fig 3.14C) were also unchanged in both *Pup1*-K46$^+$ and *Pup1*-K46$^-$ cultivars compared with LP condition ($P < 0.05$ for total chlorophyll content and $P < 0.1$ for chl b).

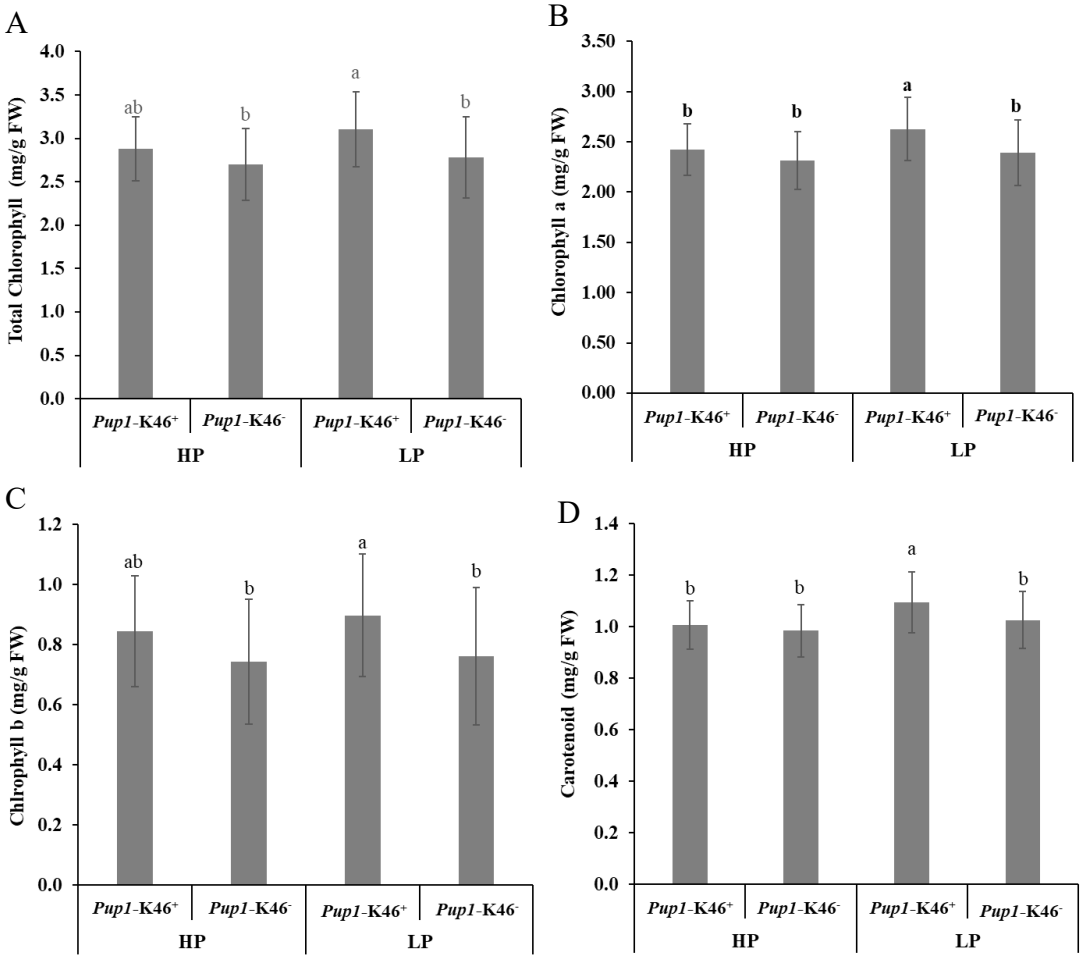


Figure 3.14 Pigment contents in the rice leaves under full-strength solution. (A) Total chlorophyll content, (B) chlorophyll a, (C) chlorophyll b, and (D) carotenoid in the *Pup1*-K46^{+} and *Pup1*-K46^{-} cultivars under the HP and LP conditions. Data correspond to the average values from 20 cultivars (n = 20). Error bars represent SD. Different letters indicate significant differences with ANOVA, LSD test; ($P \leq 0.05$).

3.3.9 Relationship between Pi content and pigment content

To determine if there are correlations between Pi content and chlorophyll content, and Pi content and carotenoid content under the LP condition. Our result showed that there were no significant correlations between the Pi content and chlorophyll a, Pi content and chlorophyll b and Pi content and carotenoid under the LP and HP conditions except for Pi content and carotenoid in *Pup1*-K46^{+} cultivars (Fig 3.15). There were no correlations between Pi content and chlorophyll a (Fig 3.15A) and between Pi content and chlorophyll b (Fig 3.15B) in the *Pup1*-K46^{+} and *Pup1*-K46^{-} cultivars, but there was a significant correlation in the Pi content and carotenoid under the HP condition in the *Pup1*-K46^{+} cultivars (Fig 3.15C). Under the LP condition, there were no significant correlations between Pi content and chlorophyll a (Fig 3.15D), Pi content and chlorophyll b (Fig 3.15E) and Pi content and chlorophyll b (Fig 3.15F) in the *Pup1*-K46^{+} and *Pup1*-K46^{-} cultivars.

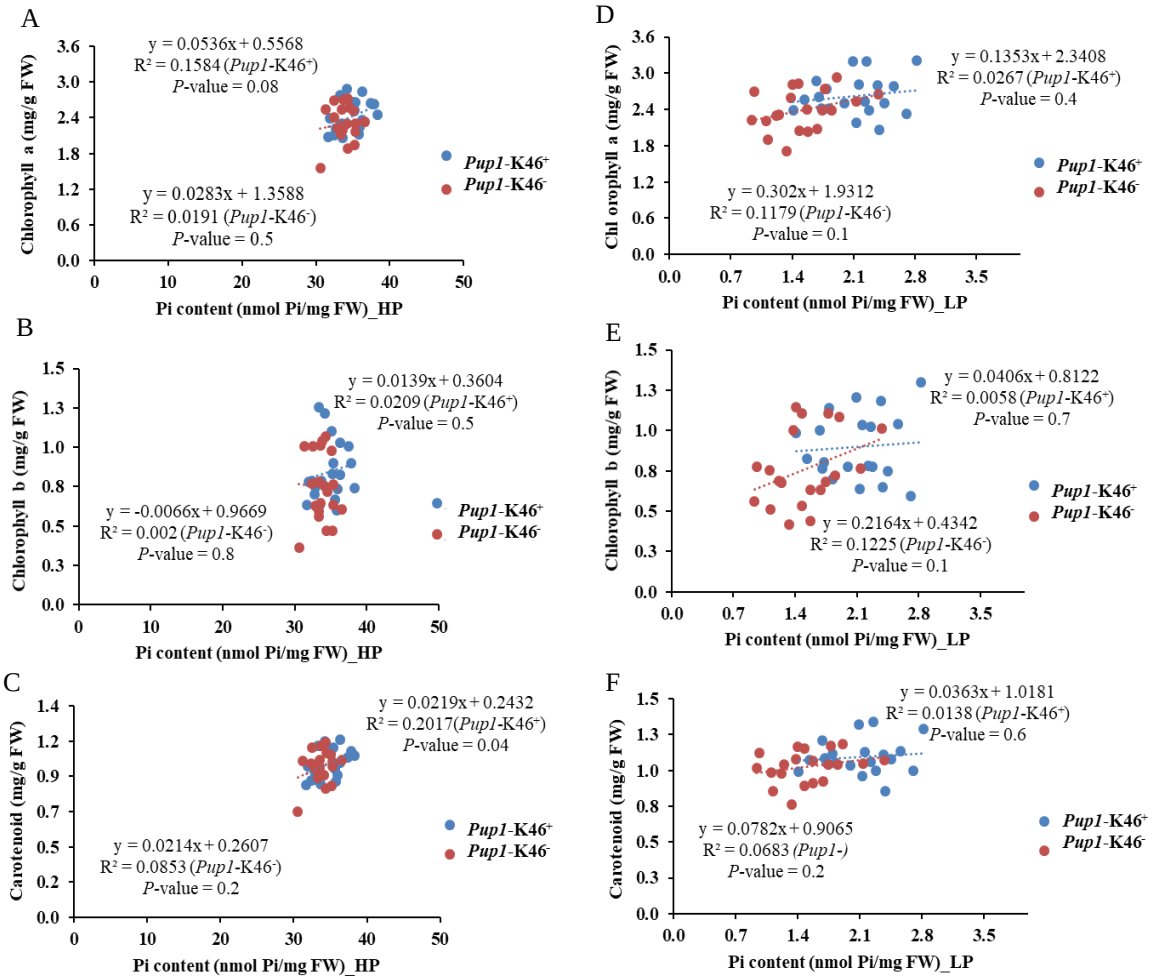


Figure 3.15 Correlations between the Pi content and pigment content in the *Pup1*-K46^{+} and *Pup1*-K46^{-} cultivars under the HP and LP conditions. Figure shows correlation between the Pi content and (A) chlorophyll a, (B) chlorophyll b, and (C) carotenoid under the HP condition, and correlation between the Pi content and (D) chlorophyll a, (E) chlorophyll b, and (F) carotenoid under the LP condition. The correlation was calculated by R software with LSD, (P-value ≤ 0.05).

CHAPTER 4
DISCUSSION

4.1 The *Pup1*-K46 marker is detected in half of the lowland rice cultivars in southern Thailand

Our study found that half of the sixty-one investigated lowland rice cultivars possessed the *Pup1*-K46 marker. Although the *Pup1*-K46 is widely distributed across the rice species of the *Oryza* genus such as *O. sativa* (Chin et al., 2011)*, O. glaberrima* (Pariasca-Tanaka et al., 2014) and *O. rufipogon* (Neelam et al., 2017), this *Pup1* gene-based marker is mostly conserved in P-deficient tolerance or upland rice cultivars and frequently absent from lowland rice cultivars (Chin et al., 2010; 2011; Pariasca-Tanaka et al., 2014). To detect its presence in the lowland rice cultivars in this study, an upland cultivar (Dular) and a lowland cultivar (IR64) were recruited as positive and negative controls)Chin et al., 2011; Gamuyao et al., 2012). From our results, the distribution of the *Pup1*-K46 region was moderately found in Thai lowland rice cultivars, supported by a previous report (Vejchasarn et al., 2016a).

4.2 The *Pup1*-K46 positive cultivars are more tolerant under P deficiency than the *Pup1*-K46 negative cultivars

The shoot dry mass reduction was significantly less in the *Pup1*-K46 positive (*Pup1*-K46^{+}) cultivars than in the *Pup1*-K46 negative (*Pup1*-K46^{-}) cultivars under the LP condition experiments in both half- and full-strength solutions. Shoot dry mass has been considered as an indicator of the P-deficient tolerance in rice since tolerant plants could maintain higher shoot biomass under such low P stress (Wissuwa & Ae, 2001; Aluwihare et al., 2016; Chankaew et al., 2019). Chankaew et al. (2019) screened 168 rice cultivars under the low P condition and reported that tolerant rice cultivars had the higher shoot biomass than the intolerant ones. Compared to Nipponbare, Kasalath as a tolerant cultivar also has higher shoot biomass under the LP condition (Vejchasarn et al., 2016b).

Moreover, shoot REP in both growth solutions was greater in the *Pup1*-K46^{+} cultivars than the *Pup1*-K46^{-} cultivars, supporting that the growth of the *Pup1*-K46^{+} cultivars is less affected by the P starvation than the *Pup1*-K46^{-} cultivars. REP is an

indicator for better plant growth under P deficiency in several other plant species. This indictor has been applied to evaluate tolerance to P shortage in wheat (Ozturk et al., 2005), coffee (Neto et al., 2016) and wild grass *Allium hookeri* Thwaites (Kshetri et al., 2018). In rice, when the *Pup1* (*PSTOL1*) was introgressed into IR64, an *indica* lowland mega cultivar (Mackill & Khush, 2018), the breeding lines had superior growth with a larger root system and greater shoot biomass under P deficiency (Gamuyao et al., 2012; Wissuwa et al., 2016). Thus, together with our results, the *Pup1*-K46^{+} cultivars possess greater tolerance to P deficiency than the *Pup1*-K46^{-} cultivars in lowland rice cultivars.

4.3 The P-deficient tolerance of the *Pup1*-K46 positive cultivars are involved with P uptake and Pi content

4.3.1 The *Pup1*-K46 positive cultivars accumulate more P in root than the *Pup1*-K46 negative cultivars under P deficiency

In both hydroponic solutions, the LP condition reduced total P concentration, and the reduction of the P concentration was more dominant in the shoot. We found that the total P concentration in the root of *Pup1*-K46^{+} cultivars was significantly higher than that of *Pup1*-K46^{-} cultivars. In rice, P deficiency directly affects total P concentration (Wissuwa et al., 2005). Under P sufficiency, P concentration is predominantly accumulated in shoots, but when P deficiency is introduced, the P concentration is favorably partitioned into roots, particularly in the tolerant rice genotypes, and this phenomenon is to improve root growth to take up more P (Wissuwa, 2003; Wissuwa et al., 2005). From our results, the *Pup1*-K46^{+} cultivars may store more P concentration into roots than the *Pup1*-K46^{-} cultivars under P deficiency.

We also found that the *Pup1*-K46^{+} cultivars took up the greater amount of P in roots than the *Pup1*-K46^{-} cultivars under the LP condition, which may be an indicative tolerance adaptation of the *Pup1*-K46^{+} cultivars toward P deficiency. P uptake is estimated from dry mass multiplied by total P concentration and is positively correlated with higher root growth (Wissuwa, 2003). Tolerant genotypes like Kasalath due to its superior root growth could uptake more P than intolerant genotype like Nipponbare (Wissuwa et al., 1998; Wissuwa & Ae, 2001). Wissuwa et al. (1998) also reported that breeding lines with the *Pup1* (*Pup1*-K46) locus could take up more P than their background parent IR64. Taken consideration into total P concentration and P uptake,

it is therefore highly possible that lowland rice cultivars with the *Pup1*-K46 could strengthen their tolerance under P limitation by absorbing more P via their roots and the presence of *Pup1*-K46 in lowland ecotype is involved with P-deficient tolerance like upland ecotype.

Under P deficiency, PUE increased in the shoot of the *Pup1*-K46^{+} cultivars in half-strength solution but in full-strength solution, increased PUE was observed in the root of the *Pup1*-K46^{-} cultivars. These contradictory results in the growing conditions could signify two possible reasons (the differences in the growing conditions explained in Section 3.3.1). First, the *Pup1*-K46^{+} cultivars might need less P under P deficiency to maintain shoot growth, and the *Pup1*-K46^{-} cultivars require less P under P deficiency to retain root growth because when P deficiency is developed, internal P is remobilized from old tissues to active tissues such as young leaves and active roots to save P consumption for survival (Rose et al., 2012). However, PUE in the preliminary experiment came from 3 of 20 cultivars, whose PUE and P concentration may not be considerable. Second, as PUE is the inverse of tissue P concentration (Rose et al., 2011), the increased PUE in the root of the *Pup1*-K46^{-} cultivars is owing to reduction of P concentration or P uptake in the root, indicating that P-deficient tolerance was mainly driven by P uptake not by PUE (Wissuwa et al., 1998). Consistent findings occurred in the *Pup1* (including *Pup1*-K46 region), the most powerful QTL for P uptake found in rice to date and mapped to the same locus as the major QTL for PUE; the *Pup1* (*Pup1*-K46) still doubled the P uptake and significantly decreased the PUE (Wissuwa & Ae, 2001). Moreover, PUE depends on the biomass produced per unit P (Rose et al., 2011), but there were no correlations found between the PUE and biomass, while the significant positive correlation between P uptake and biomass was often reported (Wissuwa et al., 1998; Wissuwa & Ae, 2001). We thus concluded that P-deficient tolerance in the *Pup1*-K46^{+} cultivars is mainly driven by P uptake rather than by PUE.

4.3.2 The *Pup1*-K46 positive cultivars deposited more Pi than the *Pup1*-K46 negative cultivars under P deficiency

We found that the LP condition reduced Pi content in the leaf (shoot) and root, and the *Pup1*-K46$^+$ cultivars showed less reduction of Pi content than the *Pup1*-K46$^-$ cultivars (Fig 3.13A; Fig S2), suggesting that Pi homeostasis of the *Pup1*-K46$^+$ cultivars seems less affected by P deficiency than the *Pup1*-K46$^-$ cultivars. Under P sufficiency, plants store most of their Pi content in the vacuole (85-95%) and the rest in the cytosol (Raghothama, 1999; Rose, 2012). However, under P deficiency, the cellular Pi content is reduced (Lee et al., 1990; Negi et al., 2016) and to regulate Pi homeostasis, plants redistribute Pi content from vacuole to cytoplasm to retain minimum cytosolic P level or from old tissues to young tissues, from shoot to root and vice versa (Raghothama, 1999). We suggest that the *Pup1*-K46$^+$ cultivars may have better performance in regulating Pi homeostasis under P deficiency than the *Pup1*-K46$^-$ cultivars, but further study is needed to confirm.

We hypothesized that the *Pup1*-K46$^+$ cultivars might recover their P level faster than the *Pup1*-K46$^-$ cultivars in the prolonged P starvation after treated with sufficient Pi for 10 hours. We found that Pi content increased under the PR, and the *Pup1*-K46$^-$ cultivars recovered their Pi content faster than the *Pup1*-K46$^+$ cultivars when exposed to Pi for 10 hours after the 4-week LP treatment**.** Rice tends to hold optimal Pi content in their tissues; it takes one-three days to reduce Pi content under the LP condition, but it takes less than a day to recover the Pi level after PR treatment (Secco et al., 2013; 2015). This may indicate that the faster Pi recovery of the *Pup1*-K46$^-$ cultivars within the short time (10 hours) is chiefly induced by higher P demand. We also found that under the PR treatment, the *Pup1*-K46$^+$ cultivars seem to translocate more Pi from root to shoot compared to *Pup1*-K46$^-$ cultivars, so the further study should focus on recovery rate over a larger time range in the two types of cultivars**.**

Previous studies reported that P deficiency reduced chlorophyll contents (Xu et al., 2007; Guo et al., 2012). However, in our study, P deficiency did not reduce the chlorophyll content by direct and indirect (SPAD) measurements. Moreover, there were no relationships between the Pi content and chlorophyll content in the leaves. Such effects of P deficiency on the reduction of the pigment contents were also observed in several other plant species such as maize (Amin et al., 2013), bean (Lima et al., 1999)

and Lupin (Passarinho et al., 2000). Under P deficiency, photosynthesis is mainly inhibited due to the reduced photosynthetic phosphorylated intermediates in the Calvin cycle, with inadequate ATP production. For instance, one molecule of Pi from cytosol is needed for every three molecules of CO_2 to make trios-Pi in the chloroplast, so reduced Pi content in the leaf adversely affects the cycle, thereby the photosynthetic rate (Pieters et al., 2001; Hernández & Munné-Bosch, 2015). Such effects have previously been reported in barley and spinach (Dietz & Foyer, 1986), soybean (Singh et al., 2018) and wheat (Rodríguez et al., 1998). In rice, P deficiency reduced the net photosynthesis (Nanamori et al., 2004; Wissuwa et al., 2005; Yong-fu et al., 2006). This indicates that the P deficiency may not affect the pigment contents and composition, but other important factors in the leaf photosynthetic systems such as net photosynthesis, stomatal conductance and transpiration rate (Xu et al., 2007; Hernández & Munné-Bosch, 2015; Veronica et al., 2017), which are not measured in this study and needed further investigation.

CHAPTER 5
CONCLUSION

In summary, we used the *Pup1*-K46 marker to diagnose the P-deficient tolerance of 61 lowland rice cultivars from southern Thailand and determine their adaptation strategies in the selected cultivars with and without the marker under P deficiency.

Half of the tested lowland rice cultivars contained the *Pup1*-K46 region in their genetic material. The LP conditions reduced the plant biomass, total P concentration, P uptake, and Pi content in the rice tissues, which did not alter the chlorophyll content in the shoots. Interestingly, the *Pup1*-K46^{+} cultivars were more tolerant to the P deficiency because the *Pup1*-K46^{+} cultivars occupied the higher efficiency for growth maintenance under both LP conditions, compared with the *Pup1*-K46^{-} cultivars.

Additionally, the *Pup1*-K46^{+} cultivars physiologically responded to the P starvation by accumulating the higher root P concentration and P uptake as well as a greater amount of Pi content in their shoot and root than the *Pup1*-K46^{-} cultivars. The *Pup1*-K46^{+} cultivars improved their capacity to deposit soluble Pi from their growth media by storing it in the cytoplasm. It is therefore confirmed that the *Pup1*-K46 region empowers lowland rice cultivars to withstand the P deficiency like the upland rice ecotype, previously reported.

REFERENCES

Aluwihare, Y. C., Ishan, M., Chamikara, M. D. M., Weebadde, C. K., Sirisena, D. N., Samarasinghe, W. L. G. and Sooriyapathirana, S. D. S. S. 2016. Characterization and selection of phosphorus deficiency tolerant rice genotypes in Sri Lanka. *Rice Science*. 23(4): 184–195.

Amin, S., Che, F. I., Hanafi, M. M., Hamed, Z. and Zaharah, A. R. 2013. Phosphorus and zinc uptake and their interaction effect on dry matter and chlorophyll content of sweet corn (*Zea mays* var. *Saccharat*). *Journal of Agronomy*. 12(4): 187–192.

Chankaew, S., Monkham, T., Pinta, W., Sanitchon, J., Kaewpradit, W. and Srinives, P. 2019. Screening tolerance to phosphorus deficiency and validation of *phosphorus uptake 1* (*Pup1*) gene-linked markers in Thai indigenous upland rice germplasm. *Agronomy*. 9(81): 1–11.

Chin, J. H., Gamuyao, R., Dalid, C., Bustamam, M., Prasetiyono, J., Moeljopawiro, S. and Heuer, S. 2011. Developing rice with high yield under phosphorus deficiency: *Pup1* sequence to application. *Plant Physiology*. 156(3): 1202–1216.

Chin, J. H., Lu, X., Haefele, S. M., Gamuyao, R., Ismail, A., Wissuwa, M. and Heuer, S. 2010. Development and application of gene-based markers for the major rice QTL *Phosphorus uptake 1*. *Theoretical and Applied Genetics*. 120(6): 1073–1086.

Dellaporta, S., Wood, J. and Hicks, J. 1983. A plant DNA minipreparation: version II. *Plant Molecular Biology Reporter*. 1(4): 19–21.

Dietz, K. J. and Foyer, C. 1986. The relationship between phosphate status and photosynthesis in leaves - Reversibility of the effects of phosphate deficiency on photosynthesis. *Planta*. 167(3): 376–381.

Dobermann, A. 2000. *Rice: Nutrient disorders & nutrient management*. IRRI.

Fageria, N. K. 2014. Yield and yield components and phosphorus use efficiency of lowland rice genotypes. *Journal of Plant Nutrition*. 37(7): 979–989.

Fageria, N. K. and Santos, A. B. 2002. Lowland rice genotypes evaluation for phosphorus use efficiency. *Journal of Plant Nutrition*. 25(12): 2793–2802.

Gamuyao, R., Chin, J. H., Pariasca-Tanaka, J., Pesaresi, P., Catausan, S., Dalid, C. and Heuer, S. 2012. The protein kinase Pstol1 from traditional rice confers tolerance

of phosphorus deficiency. *Nature*. 488: 535–539.

Guo, T. R., Yao, P. C., Zhang, Z. D., Wang, J. J. and Wang, M. 2012. Involvement of antioxidative defense system in rice seedlings exposed to aluminum toxicity and phosphorus deficiency. *Rice Science*. 19(3): 207–212.

Hernández, I. and Munné-Bosch, S. 2015. Linking phosphorus availability with photo-oxidative stress in plants. *Journal of Experimental Botany*. 66(10): 2889–2900.

Heuer, S., Lu, X., Chin, J. H., Tanaka, J. P., Kanamori, H., Matsumoto, T. and Wissuwa, M. 2009. Comparative sequence analyses of the major quantitative trait locus *phosphorus uptake 1* (*Pup1*) reveal a complex genetic structure. *Plant Biotechnology Journal*. 7(5): 456–471.

Hochholdinger, F., Park, W. J., Sauer, M. and Woll, K. 2004. From weeds to crops: Genetic analysis of root development in cereals. *Trends in Plant Science*. 9(1): 42–48.

Hopkins, B. 2015. *Phosphorus: Handbook of Plant Nutrition,* Second Edition. 65-126. CRC Press.

Hu, X., Tanaka, A. and Tanaka, R. 2013. Simple extraction methods that prevent the artifactual conversion of chlorophyll to chlorophyllide during pigment isolation from leaf samples. *Plant Methods*. 9(19): 1–13.

Hurry, V., Strand, A., Furbank, R. and Stitt, M. 2000. The role of inorganic phosphate in the development of freezing tolerance and the acclimatization of photosynthesis to low temperature is revealed by the pho mutants of *Arabidopsis thaliana*. *The Plant Journal*. 24(3): 383–396.

Johri, A. K., Oelmüller, R., Dua, M., Yadav, V., Kumar, M., Tuteja, N. and Stroud, R. M. 2015. Fungal association and utilization of phosphate by plants: Success, limitations, and future prospects. *Frontiers in Microbiology*. 6: 984.

Kaewubon, P., Hutadilok-Towatana, N., Teixeira da Silva, J. A. and Meesawat, U. 2015. Ultrastructural and biochemical alterations during browning of pigeon orchid (*Dendrobium crumenatum* Swartz) callus. *Plant Cell, Tissue and Organ Culture*. 121(1): 53–69.

Kant, J., Ishizaki, T., Pariasca-Tanaka, J., Rose, T., Wissuwa, M. and Watt, M. 2018.

Phosphorus efficient phenotype of rice. *Rice Crop - Current Developments*. 129.

Kondo, M., Pablico, P. P., Aragones, D. V., Agbisit, R., Abe, J., Morita, S. and Courtois, B. 2003. Genotypic and environmental variations in root morphology in rice genotypes under upland field conditions. *Plant and Soil*. 255(1): 189–200.

Kshetri, L., Pandey, P. and Sharma, G. D. 2018. Rhizosphere mediated nutrient management in *Allium hookeri* thwaites by using phosphate solubilizing rhizobacteria and tricalcium phosphate amended soil. *Journal of Plant Interactions.* 13(1): 256–269.

Lan, Z. M., Lin, X. J., Wang, F., Zhang, H. and Chen, C. R. 2012. Phosphorus availability and rice grain yield in a paddy soil in response to long-term fertilization. *Biology and Fertility of Soils*. 48(5): 579–588.

Lee, R. B., Ratcliffe, R. G. and Southon, T. E. 1990. 31P NMR measurements of the cytoplasmic and vacuolar Pi content of mature maize roots: Relationships with phosphorus status and phosphate fluxes. *Journal of Experimental Botany*. 41(9): 1063–1078.

Lima, J. D., Mosquim, P. R. and Da Matta, F. M. 1999. Leaf gas exchange and chlorophyll fluorescence parameters in phaseolus vulgaris. *Photosynthetica*. 37(1): 113-121.

Liu, F., Chang, X. J., Ye, Y., Xie, W. B., Wu, P. and Lian, X. M. 2011. Comprehensive sequence and whole-life-cycle expression profile analysis of the phosphate transporter gene family in rice. *Molecular Plant*. 4(6): 1105–1122.

Lynch, J. P. 2019. Root phenotypes for improved nutrient capture: an underexploited opportunity for global agriculture. *New Phytologist*. 223: 548-564.

Lynch, J. P. and Brown, K. M. 2001. Topsoil foraging–an architectural adaptation of plants to low phosphorus availability. *Plant and Soil*. 237(2): 225-237.

Mackill, D. J. and Khush, G. S. 2018. IR64: a high-quality and high-yielding mega variety. *Rice*. 11(1): 18.

Maclean, J., Hardy, B. and Hettel, G. 2013. *Rice Almanac: Source book for one of the most important economic activities on earth*. IRRI.

Maksup, S., Supaibulwatana, K. and Selvaraj, G. 2013. High-quality reference genes

for quantifying the transcriptional responses of *Oryza sativa* L. (ssp. *indica* and *japonica*) to abiotic stress conditions. *Chinese Science Bulletin*. 58(16): 1919–1930.

Morita, S. and Nemoto, K. 1995. Morphology and anatomy of rice roots with special reference to coordination in organo- and histogenesis. In F. Baluska et al. (eds.), *Structure and Function of Roots*. 75–86.

Nagumo, T., Tajima, S., Chikushi, S. and Yamashita, A. 2013. Phosphorus balance and soil phosphorus status in paddy rice fields with various fertilizer practices. *Plant Production Science*. 16(1): 69–76.

Nanamori, M., Shinano, T., Wasaki, J., Yamamura, T., Rao, I. M. and Osaki, M. 2004. Low phosphorus tolerance mechanisms: Phosphorus recycling and photosynthate partitioning in the tropical forage grass, *Brachiaria* hybrid cultivar Mulato compared with rice. *Plant and Cell Physiology*. 45(4): 460–469.

Neelam, K., Thakur, S., Neha, Yadav, I. S., Kumar, K., Dhaliwal, S. S. and Singh, K. 2017. Novel alleles of phosphorus-starvation tolerance 1 gene (*PSTOL1*) from *Oryza rufipogon* confers high phosphorus uptake efficiency. *Frontiers in Plant Science*. 8: 509.

Negi, M., Sanagala, R., Rai, V. and Jain, A. 2016. Deciphering phosphate deficiency-mediated temporal effects on different root traits in rice grown in a modified hydroponic system. *Frontiers in Plant Science.* 7: 550.

Neto, A. P., Favarin, J. L., Tezotto, T., Couto, H. T. Z. and Hammond, J. P. 2016. Analysis of phosphorus use efficiency traits in coffea genotypes reveals *Coffea arabica* and *Coffea canephora* have contrasting phosphorus uptake and utilization efficiencies. *Frontiers in Plant Science*. 7: 408.

O'toole, J. C. and Bland, W. L. 1987. Genotypic variation in crop plant root systems. *Advances in Agronomy*. 41: 91-145. Academic Press.

Ozturk, L., Eker, S., Torun, B. and Cakmak, I. 2005. Variation in phosphorus efficiency among 73 bread and durum wheat genotypes grown in a phosphorus-deficient calcareous soil. *Plant and Soil*. 269: 69–80.

Pariasca-Tanaka, J., Chin, J. H., Dramé, K. N., Dalid, C., Heuer, S. and Wissuwa, M. 2014. A novel allele of the P-starvation tolerance gene *OsPSTOL*1 from African

rice (*Oryza glaberrima* Steud) and its distribution in the genus *Oryza*. *Theoretical and Applied Genetics*. 127: 1387–1398.

Passarinho, J. A., Rodrigues, M. L., Osório, M. L., Ricardo, C. P. P. and Chaves, M. M. 2000. Physiological responses of *Lupinus mutabilis* to phosphorus nutrition and season of growth. *Journal of Plant Nutrition*. 23(4): 487–505.

Phimsirikul, P. and Matoh, T. 2003. The status of phosphorus in Thai soils and P evaluation using EDTA-NaF extraction method EDTA-NaF. *Songklanakarin Journal of Science and Technology*. 25(4): 423–434.

Pieters, A. J., Paul, M. J. and Lawlor, D. W. 2001. Low sink demand limits photosynthesis under Pi deficiency. *Journal of Experimental Botany*. 52(358): 1083–1091.

Raghothama, K. G. 1999. Phosphate acquisition. *Annu.Rev. Plant Physiol.* 50: 665–93.

Rebouillat, J., Dievart, A., Verdeil, J. L., Escoute, J., Giese, G., Breitler, J. C. and Périn, C. 2009. Molecular genetics of rice root development. *Rice*. 2(1): 15–34.

Reich, M., Aghajanzadeh, T. and De Kok, L. 2014. Physiological basis of plant nutrient use efficiency – concepts, opportunities and challenges for its improvement. *Nutrient Use Efficiency in Plants*. 10: 1–27.

Rodríguez, D., Pomar, M. C. and Goudriaan, J. 1998. Leaf primordia initiation, leaf emergence and tillering in wheat (*Triticum aestivum L.*) grown under low-phosphorus conditions. *Plant and Soil*. 202(1): 149–157.

Rose, T. J., Rose, M. T., Pariasca-Tanaka, J., Heuer, S. and Wissuwa, M. 2011. The Frustration with Utilization: Why have improvements in internal phosphorus utilization efficiency in crops remained so elusive?. *Frontiers in Plant Science*. 2: 73.

Rose, T. J. and Wissuwa, M. 2012. Rethinking internal phosphorus utilization efficiency. a new approach is needed to improve pue in grain crops. *Advances in Agronomy*. 116: 185–217. Academic Press.

Saleque, M. A., Abedin, M. J., Panaullah, G. M. and Bhuiyan, N. I. 1998. Yield and phosphorus efficiency of some lowland rice varieties at different levels of soil-available phosphorus. *Communications in Soil Science and Plant Analysis*.

29(19&20): 2905–2916.

Secco, D., Jabnoune, M., Walker, H., Shou, H., Wu, P., Poirier, Y. and Whelan, J. 2013. Spatio-temporal transcript profiling of rice roots and shoots in response to phosphate starvation and recovery. *The Plant Cell*. 25(11): 4285–4304.

Secco, D., Wang, C., Shou, H., Schultz, M. D., Chiarenza, S., Nussaume, L. and Lister, R. 2015. Stress induced gene expression drives transient DNA methylation changes at adjacent repetitive elements. *Plant Biology*. 4: 1–26.

Shi, J., Hu, H., Zhang, K., Zhang, W., Yu, Y., Wu, Z. and Wu, P. 2014. The paralogous SPX3 and SPX5 genes redundantly modulate Pi homeostasis in rice. *Journal of Experimental Botany.* 65(3): 859–870.

Singh, S. K., Reddy, V. R., Fleisher, D. H. and Timlin, D. J. 2018. Phosphorus nutrition affects temperature response of soybean growth and canopy photosynthesis. *Frontiers in Plant Science*. 9.

Ticconi, C. A., Delatorre, C. A., Lahner, B., Salt, D. E. and Abel, S. 2004. Arabidopsis pdr2 reveals a phosphate-sensitive checkpoint in root development. *The Plant Journal.* 37(6): 801-814.

Uwasawa, M., Sangtong, P., Sangtong, P. and Cholitkul, W. 1988. Behavior of phosphorus in paddy soils of Thailand. *Soil Science and Plant Nutrition*. 34(1): 41–53.

van de Wiel, C. C. M., van der Linden, C. G. and Scholten, O. E. 2016. Improving phosphorus use efficiency in agriculture: opportunities for breeding. *Euphytica*. 207: 1–22.

Vejchasarn, P., Kotchasatit, U., Jairin, J., Wongboon, W., Wongnongwa, J., Phansenee, Y., Kawichai, R., Leelakud, P. and Chamarerk, V. 2016a. Physiological and morphological responses of rice to low phosphorus availability. *Thai Rice Research Journal*. 7(1): 74–83.

Vejchasarn, P., Lynch, J. P. and Brown, K. M. 2016b. Genetic variability in phosphorus responses of rice root phenotypes. *Rice*. 9: 29.

Veronica, N., Subrahmanyam, D., Vishnu Kiran, T., Yugandhar, P., Bhadana, V. P., Padma, V. and Voleti, S. R. 2017. Influence of low phosphorus concentration on

leaf photosynthetic characteristics and antioxidant response of rice genotypes. *Photosynthetica*. 55(2): 285–293.

Wang, K., Cui, K., Liu, G., Xie, W., Yu, H., Pan, J. and Peng, S. 2014. Identification of quantitative trait loci for phosphorus use efficiency traits in rice using a high density SNP map. *BMC Genetics*. 15(155): 1–15.

Wissuwa, M. 2003. How do plants achieve tolerance to phosphorus deficiency? small causes with big effects. *Plant Physiology*. 133(4): 1947–1958.

Wissuwa, M. and Ae, N. 2001. Genotypic variation for tolerance to phosphorus deficiency in rice and the potential for its exploitation in rice improvement. *Plant Breeding*. 120: 43–48.

Wissuwa, M., Gamat, G. and Ismail, A. M. 2005. Is root growth under phosphorus deficiency affected by source or sink limitations?. *Journal of Experimental Botany*. 56(417): 1943–1950.

Wissuwa, M., Kretzschmar, T. and Rose, T. J. 2016. From promise to application: root traits for enhanced nutrient capture in rice breeding. *Journal of Experimental Botany*. 67(12): 3605–3615.

Wissuwa, M., Wegner, J., Ae, N. and Yano, M. 2002. Substitution mapping of *Pup1*: a major QTL increasing phosphorus uptake of rice from a phosphorus-deficient soil. *Theoretical and Applied Genetics*. 105: 890-897.

Wissuwa, M, Yano, M. and Ae, N. 1998. Mapping QTLs for phosphorus deficiency tolerance in rice (*Oryza sativa L.*). *Theoretical and Applied Genetics*. 97: 777–783.

Xu, H. X., Weng, X. Y. and Yang, Y. 2007. Effect of phosphorus deficiency on the photosynthetic characteristics of rice plants. *Russian Journal of Plant Physiology.* 54(6): 741–748.

Yan, C., Zhan, H., Yan, S., Dong, S., Ma, C., Song, Q., Gong, Z. and Barbie, M. 2016. Effects of straw retention and phosphorous fertilizer application on available phosphorus content in the soil solution during rice growth. *Paddy and Water Environment*. 14(1): 61–69.

Yi, K., Wu, Z., Zhou, J., Du, L., Guo, L., Wu, Y. and Wu, P. 2005. OsPTF1, a novel transcription factor involved in tolerance to phosphate starvation in rice. *Plant*

Physiology. 138: 2087–2096.

Yong-fu, L., An-cheng, L., Assan, M. J. H. and Xing-hua, W. 2006. Effect of phosphorus deficiency on leaf photosynthesis and carbohydrates partitioning in two rice genotypes with contrasting low phosphorus susceptibility. *Rice Science*. 13(4): 283–290.

Yoshida, S., Forno, Douglas A., Cock, James H., and Gomez, K. A. 1976. *Laboratory manual for physiological studies of rice*. The International Rice Research Institute (3rd ed.). Manila, The Philippines.

Zeigler, R. and Puckridge, D. 1995. Improving sustainable productivity in rice-based rainfed lowland systems of south and southeast asia. *GeoJournal*. 35(3): 307–324.

APPENDICES

Appendix A

List of cultivars

Table S1 List of *Pup1*-K46$^+$ and *Pup1*-K46$^-$ cultivars used in the preliminary experiment and the main experiments.

Preliminary experiment		Main Experiment	
Pup-K46$^+$	*Pup*-K46$^-$	*Pup*-K46$^+$	*Pup*-K46$^-$
Khao Leuang	Chana	Buang Khao	Chana
Buang Khao	Chuk Tium Dang	Chaw La Mai	Gan Tang
Hawm Bai-Tuey	Dawk Payorm	Eaw Mod Daeng	Khao Rak
Hawm Nil	Gan Tang	Hawm Bai-Tuey	Khem Thawng
Hawm Tammasat	Khem Thawng	Hawm Tammasat	Klah Nak
Hua Nadam	Klah Nak	Hua Nadam	Glib Mek
Hal Ham Doo Lila	Glib Mek	Hal Ham Doo Lila	Look Khuey
KDML105	Khom	KDML105	Look Plah
Kaen Chan	Look Plah	Kaen Chan	Look Taw
Koo Ning	Look Taw	Koo Ning	Malay
Look Dam	Malay	Leb Nok	Mlea-Hawm
Lam Hak	Mlea-Hawm	Look Dam	Man Tom
Ma Jah Nu	Man Tom	Lam Hak	Nahng Loi
Mae Mai	Nahg Loi	Mae Mai	Poo Yoo
Meuang Sai	Poo Yoo	Meung Sai	Puang Wai
Maw Arun	Puang Wai	Maw Arun	Rai Sai
Pathumthani 1	Rai Sai	Pathumthani 1	RD 41
Ra den	RD 41	RD15	RD61
Sahn Suay	RD61	Sahn Suay	Sae Mah
Sang Yod	Sae Mah	Sang Yod	Tia Malay-Daeng
	Tia Malay-Daeng		

Appendix B
Hydroponic solution

Table S2 Preparation of stock solution and components for the half-strength Yoshida solution. To make stock solution, each chemical was dissolved in distilled water (5 l). The stock number 6 was dissolved separately, then mixed with 250 ml 1M H_2SO_4 and finally added distilled water up to 5 l. To make 20 l HP solution, 12.5 ml of each stock solution from number 1 to 6 were mixed; this HP condition contained 5 mg/l P. For 20 l LP solution, 12.5 ml of each stock solution from number 2 to 7 were mixed; this LP solution contained 0.25 mg/l P.

Stock number	Chemical	Main element	Amount (g) /5 l	Amount of stock solution needed for 20 l	Concentration of the element in working solution (mg/l)
1	$NaH_2PO_4.H_2O$	P (HP)	201.5 g	12.5 ml	5
2	NH_4NO_3	N	457 g	12.5 ml	20
3	K_2SO_4	K	357 g	12.5 ml	20
4	$CaCl_2.2H_2O$	Ca	586.82 g	12.5 ml	20
5	$MgSO_4.7H_2O$	Mg	1620 g	12.5 ml	20
6	$MnCl_2.4H_2O$	Mn	7.5 g	12.5 ml	0.5
	$(NH_4)_6Mo_7O_{24}.4H_2O$	Mo	0.37 g		0.25
	H_3BO_3	B	4.67 g		0.1
	$ZnSO_4.7H_2O$	Zn	0.175 g		0.005
	$CuSO_4.5H_2O$	Cu	0.155 g		0.005
	Ferric sodium EDTA	Fe	51.6 g		1
	Citric acid $C_6H_8O_7.H_2O$		59.5 g		
	1M H_2SO_4		250 ml		
7	$NaH_2PO_4.H_2O$	P (LP)	10 g	12.5 ml	0.25 (Yi et al., 2005)

Table S3 Preparation of stock solution and components for the full-strength Yoshida solution. To make stock solution, each chemical was dissolved in distilled water (5 l). The stock number 6 was dissolved separately, then mixed with 250 ml 1M H_2SO_4 and finally added distilled water up to 5 l. To make 20 l HP solution, 25 ml of each stock solution from number 1 to 6 were mixed; this HP condition contained 10 mg/l P. For 20 l LP solution, 25 ml of each stock solution from number 2 to 7 were mixed; this LP solution contained 0.5 mg/l P.

Stock number	Chemical	Main element	Amount (g) /5 l	Amount of stock solution needed for 20 l	Concentration of the element in working solution (mg/l)
1	$NaH_2PO_4.H_2O$	P (HP)	201.5 g	25 ml	10
2	NH_4NO_3	N	457 g	25 ml	40
3	K_2SO_4	K	357 g	25 ml	40
4	$CaCl_2.2H_2O$	Ca	586.82 g	25 ml	40
5	$MgSO_4.7H_2O$	Mg	1620 g	25 ml	40
6	$MnCl_2.4H_2O$	Mn	7.5 g	25 ml	0.5
	$(NH_4)_6Mo_7O_{24}.4H_2O$	Mo	0.37 g		0.05
	H_3BO_3	B	4.67 g		0.2
	$ZnSO_4.7H_2O$	Zn	0.175 g		0.01
	$CuSO_4.5H_2O$	Cu	0.155 g		0.01
	Ferric sodium EDTA	Fe	51.6 g		2
	Citric acid $C_6H_8O_7.H_2O$		59.5 g		
	1M H_2SO_4		250 ml		
7	$NaH_2PO_4.H_2O$	P (LP)	10 g	25 ml	0.5 (Yi et al., 2005)

Appendix C

Supplementary data

Table S4 Biomass of the *Pup1*-K46$^+$ and *Pup1*-K46$^-$ rice cultivars under half-strength Yoshida solution (n = 20 and 21)

Cultivars	Shoot Dry Weight			Root Dry Weight		
	HP	LP	*P*-value	HP	LP	*P*-value
Pup1-K46$^+$						
Khao Leuang	41.50±9.55	38.59±6.46	0.254	10.18±2.85	10.18±1.75	1.000
Kaen Chan	50.23±6.22	43.59±8.63	0.007	15.73±2.11	14.59±2.99	0.163
Mae Mai	49.59±6.93	43.68±3.48	0.001	13.95±2.20	13.18±1.53	0.194
Hal Ham Doo Lila	44.00±5.52	38.64±6.73	0.007	11.50±1.95	10.27±2.30	0.069
Hua Nadam	45.27±4.48	44.41±4.28	0.527	14.14±1.63	13.18±1.77	0.077
Buang Khao	46.64±4.87	44.91±4.37	0.233	11.23±1.62	11.82±1.53	0.231
Ma Jah Nu	39.55±6.15	38.55±3.64	0.525	11.95±1.92	11.72±1.21	0.649
Lam Hak	28.95±7.19	28.18±8.36	0.750	8.27±2.61	8.45±3.19	0.841
Ra den	39.55±7.67	34.59±8.84	0.059	11.32±2.18	10.00±3.03	0.113
Look Dam	38.38±5.84	37.29±5.18	0.732	11.62±1.81	12.14±1.86	0.234
Koo Ning	27.27±7.36	25.55±7.34	0.451	8.36±1.94	7.23±2.57	0.114
Meuang Sai	26.91±10.14	24.68±10.34	0.485	9.50±2.62	8.09±3.36	0.137
Maw Arun	35.44±11.49	33.44±9.81	0.589	10.23±3.28	9.28±3.19	0.374
Sahn Suay	44.64±14.78	39.41±12.71	0.226	12.27±3.78	10.64±3.89	0.174
Sang Yod	31.86±8.72	31.50±6.97	0.882	8.36±2.19	8.59±2.52	1.000
Hawm Nil	32.05±13.42	28.55±11.66	0.372	10.68±3.56	10.18±3.55	0.651
Hawm Bai-Tuey	51.64±12.94	50.82±12.86	0.838	13.27±3.69	13.32±3.64	0.968
Hawm Tammasat	44.47±11.62	43.37±9.17	0.753	12.16±2.70	12.26±3.04	0.913
Pathumthani 1	39.11±11.59	38.84±9.51	0.941	16.00±5.07	14.05±3.87	0.203
KDML105	50.00±14.32	48.77±14.23	0.784	13.86±4.15	13.55±3.16	0.784
Average±SD	40.62±7.56	37.87±7.20	0.311	11.14±2.10	11.73±2.21	0.400
Pup1-K46$^-$						
Chuk Tium Dang	35.82±5.13	35.41±4.56	0.786	13.86±1.89	12.55±2.46	0.058
Look Taw	35.04±4.47	34.23±4.43	0.554	11.77±1.56	11.73±2.22	0.939
Chana	34.64±5.24	32.59±4.61	0.186	11.50±1.70	10.95±1.77	0.314
Man Tom	39.36±5.71	35.59±12.59	0.218	11.68±1.94	11.09±3.74	0.524
Dawk Payorm	33.68±6.37	29.45±7.00	0.047	13.32±3.02	12.23±3.57	0.291
Khom	37.86±13.43	30.45±11.66	0.063	11.55±4.47	8.95±3.67	0.046
Rai Sai	28.68±11.36	23.36±6.46	0.069	9.18±3.75	7.41±2.23	0.070
Poo Yoo	32.70±10.88	30.75±8.25	0.537	9.10±3.27	8.80±2.64	0.757
Nahng Loi	33.65±7.72	29.53±7.56	0.137	11.35±3.14	10.88±2.76	0.656
Puang Wai	37.59±10.62	35.41±9.01	0.477	9.18±3.42	8.41±2.48	0.407
Gan Tang	47.23±19.87	37.27±16.11	0.082	11.50±5.17	9.86±4.34	0.273
Glib Mek	35.73±7.15	31.91±7.19	0.092	9.59±2.72	8.23±2.25	0.085
Klah Nak	36.55±8.41	34.60±10.20	0.524	14.20±2.99	13.45±4.06	0.521
Sae Mah	48.18±8.48	42.23±6.71	0.015	13.59±1.92	14.23±2.11	0.313
Tia Malay-Daeng	36.47±7.52	34.29±7.27	0.412	10.06±2.01	11.88±2.42	0.003
Khem Thawng	49.27±8.44	41.68±5.31	0.001	13.32±2.51	13.32±2.36	1.000
Look Plah	33.18±7.91	31.54±6.13	0.458	7.86±2.05	7.73±2.20	0.836
Malay	42.50±10.19	37.18±7.99	0.067	10.32±2.94	10.09±2.89	0.802
RD 41	42.45±8.13	39.73±6.14	0.227	12.41±2.42	12.23±2.25	0.803
Mlea-Hawm	40.05±6.45	38.86±8.92	0.631	11.29±2.19	11.90±3.08	0.468
RD61	44.26±9.16	40.74±8.35	0.235	12.00±2.90	11.47±2.85	0.587
Average±SD	38.33±5.39	34.61±4.57	0.023	11.36±1.71	10.83±1.93	0.358

Table S4 Biomass of the *Pup1*-K46^{+} and *Pup1*-K46^{-} rice cultivars under half-strength Yoshida solution (n = 20 and 21) (continued)

Cultivars	Total Dry Weight			Root/shoot ratio		
	HP	LP	*P*-value	HP	LP	*P*-value
Pup1-K46^{+}						
Khao Leuang	51.68±12.10	48.77±7.87	0.361	0.25±0.04	0.27±0.04	0.078
Kaen Chan	65.95±7.99	58.18±10.52	0.010	0.31±0.03	0.35±0.10	0.161
Mae Mai	63.55±8.96	56.86±4.66	0.004	0.28±0.02	0.30±0.03	0.007
Hal Ham Doo Lila	55.50±7.25	48.91±8.80	0.011	0.26±0.03	0.27±0.03	0.603
Hua Nadam	59.41±5.91	57.59±5.83	0.321	0.31±0.02	0.30±0.02	0.026
Buang Khao	57.86±6.23	56.73±5.58	0.537	0.24±0.02	0.26±0.02	0.002
Ma Jah Nu	51.50±7.85	50.27±4.54	0.539	0.30±0.03	0.30±0.02	0.802
Lam Hak	37.23±9.62	36.64±11.52	0.858	0.28±0.04	0.29±0.04	0.481
Ra den	50.86±9.66	44.59±11.77	0.066	0.29±0.03	0.29±0.03	0.982
Look Dam	50.00±7.16	49.43±6.62	0.966	0.31±0.04	0.33±0.04	0.078
Koo Ning	35.64±9.16	32.77±9.82	0.334	0.31±0.05	0.28±0.04	0.014
Meuang Sai	36.41±12.56	32.77±13.64	0.374	0.37±0.08	0.33±0.04	0.027
Maw Arun	45.72±14.71	42.72±12.89	0.207	0.29±0.03	0.28±0.03	0.031
Sahn Suay	56.91±18.36	50.04±16.31	0.012	0.28±0.04	0.27±0.06	0.036
Sang Yod	40.23±10.77	40.09±9.37	0.386	0.27±0.02	0.267±0.03	0.025
Hawm Nil	42.73±16.87	38.72±15.10	0.423	0.35±0.07	0.37±0.06	0.296
Hawm Bai-Tuey	64.91±16.25	64.14±16.29	0.878	0.26±0.04	0.26±0.03	0.574
Hawm Tammasat	56.63±14.00	55.63±12.01	0.819	0.28±0.05	0.28±0.03	0.947
Pathumthani 1	55.10±16.44	52.89±13.10	0.658	0.41±0.05	0.36±0.05	0.004
KDML105	63.86±17.70	62.32±16.99	0.777	0.28±0.05	0.28±0.05	0.853
Average±SD	52.08±9.46	49.00±9.14	0.314	0.30±0.04	0.30±0.03	0.966
Pup1-K46^{-}						
Chuk Tium Dang	49.68±6.55	47.95±5.38	0.356	0.39±0.05	0.36±0.09	0.167
Look Taw	46.82±5.76	45.95±6.41	0.648	0.34±0.03	0.34±0.04	0.688
Chana	46.14±6.74	43.55±6.18	0.201	0.33±0.03	0.33±0.03	0.786
Man Tom	51.05±7.38	46.68±16.16	0.267	0.30±0.03	0.32±0.05	0.072
Dawk Payorm	47.00±9.16	41.68±10.44	0.086	0.39±0.04	0.41±0.04	0.196
Khom	49.41±17.72	39.41±15.21	0.056	0.30±0.03	0.29±0.04	0.328
Rai Sai	37.86±14.97	30.77±8.54	0.066	0.32±0.04	0.32±0.04	0.738
Poo Yoo	41.80±13.94	39.55±10.66	0.580	0.28±0.04	0.29±0.05	0.692
Nahng Loi	45.00±10.68	40.41±10.20	0.223	0.34±0.03	0.37±0.04	0.010
Puang Wai	46.77±13.90	43.82±10.87	0.447	0.24±0.03	0.24±0.04	0.994
Gan Tang	58.73±24.85	47.14±20.34	0.106	0.24±0.03	0.26±0.03	0.033
Glib Mek	45.32±9.69	40.14±9.30	0.084	0.26±0.04	0.26±0.03	0.439
Klah Nak	50.75±11.21	48.05±14.11	0.518	0.39±0.04	0.39±0.04	0.978
Sae Mah	61.77±9.76	56.45±8.58	0.068	0.29±0.04	0.34±0.03	< 0.001
Tia Malay-Daeng	46.53±9.22	46.18±9.37	0.183	0.28±0.04	0.35±0.05	0.064
Khem Thawng	62.59±10.78	55.00±7.56	0.965	0.27±0.02	0.32±0.02	< 0.001
Look Plah	41.05±9.74	39.27±8.19	0.136	0.24±0.03	0.24±0.03	0.727
Malay	52.82±12.92	47.27±10.62	0.860	0.24±0.03	0.27±0.04	0.018
RD 41	54.86±10.26	51.95±7.99	0.311	0.30±0.03	0.31±0.04	0.202
Mlea-Hawm	51.33±8.28	50.76±11.76	0.107	0.28±0.03	0.31±0.04	0.049
RD61	56.26±11.98	52.21±11.04	0.430	0.27±0.02	0.28±0.03	0.210
Average±SD	49.69±6.34	45.44±5.97	0.034	0.30±0.05	0.31±0.05	0.333

Table S5 SPAD values measured by chlorophyll meter on rice leaves of *Pup1*-K46^{+} and *Pup1*-K46^{-} cultivars under half-strength Yoshida solution (n = 20 and 21)

Cultivars	SPAD value (Chl content)		Cultivars	SPAD value (Chl content)	
	HP	LP		HP	LP
Pup1-K46^{+}			*Pup1*-K46^{-}		
Khao Leuang	24.85±1.75	25.40±1.89	Chuk Tium Dang	22.89±1.35	24.68±1.33
Kaen Chan	23.46±0.64	24.17±1.68	Look Taw	19.92±0.73	21.85±1.35
Mae Mai	24.80±0.64	26.32±1.68	Chana	22.32±0.57	24.15±0.86
Hal Ham Doo Lila	21.00±0.51	22.41±0.92	Man Tom	23.75±1.33	24.86±1.50
Hua Nadam	22.66±0.96	23.63±0.64	Dawk Payorm	22.52±1.23	20.00±1.67
Buang Khao	21.65±1.15	23.28±0.96	Khom	20.08±1.64	23.26±0.81
Ma Jah Nu	20.86±0.66	22.70±1.41	Rai Sai	23.05±1,68	23.48±2.87
Lam Hak	22.90±0.77	24.77±0.97	Poo Yoo	22.67±1.11	23.51±1.07
Ra den	22.77±1.12	23.28±1.01	Nahng Loi	22.76±0.59	24.48±1.51
Look Dam	24.08±0.68	25.18±0.78	Puang Wai	21.66±1.28	23.94±1.06
Koo Ning	22.47±1.07	22.98±1.66	Gan Tang	22.49±1.56	23.82±1.71
Meuang Sai	21.04±1.39	23.87±1.43	Glib Mek	22.24±1.53	23.06±1.52
Maw Arun	21.85±0.71	24.77±1.06	Klah Nak	22.53±1.25	25.08±1.03
Sahn Suay	24.03±2.11	25.39±1.96	Sae Mah	21.41±1.32	22.63±1.12
Sang Yod	21.75±1.23	23.11±0.87	Tia Malay-Daeng	22.06±1.34	23.91±1.25
Hawm Nil	24.85±1.76	26.49±1.96	Khem Thawng	22.31±1.13	23.25±1.14
Hawm Bai-Tuey	24.57±1.35	26.66±2.12	Look Plah	23.13±1.53	24.33±0.87
Hawm Tammasat	23.89±1.17	26.34±0.70	Malay	22.02±1.40	23.43±1.16
Pathumthani 1	25.66±1.14	27.91±1.01	RD 41	23.85±0.85	26.02±1.05
KDML105	24.26±1.08	24.85±1.44	Mlea-Hawm	22.56±0.90	24.49±1.52
			RD61	26.18±1.01	26.65±1.74
Average±SD	23.17±1.44	24.68±1.50	Average±SD	22.50±1.25	23.85±1.36
P-value	0.003		*P*-value	0.002	

.

Table S6 Biomass of the *Pup1*-K46^{+} and *Pup1*-K46^{-} rice cultivars in full-strength Yoshida solution (n = 20)

Cultivars	Shoot dry weight (mg)			Root dry weight (mg)		
	HP	LP	*P*-values	HP	LP	*P*-values
Pup1-K46^{+}						
Kaen Chan	78.75±6.65	78.75±7.24	1.000	15.50±3.04	22.08±2.87	<0.001
Mae Mai	77.83±7.79	73.50±3.69	0.109	16.25±2.20	20.42±2.36	< 0.001
Hal Ham Doo Lila	65.17±7.81	64.92±5.75	0.933	13.50±1.94	16.92±1.89	< 0.001
Chaw La Mai	94.00±14.32	80.50±7.01	0.010	24.17±5.74	17.25±2.98	0.002
Hua Nadam	78.75±7.57	78.58±5.71	0.954	17.17±3.18	23.42±2.40	< 0.001
Buang Khao	91.25±5.60	77.50±6.49	< 0.001	16.58±2.36	16.58±1.32	1.000
Eaw Mod Daeng	80.33±9.10	72.67±3.32	0.015	27.50±6.05	20.08±3.09	0.002
Lam Hak	70.25±16.2	60.50±9.13	0.093	13.67±3.40	17.00±3.32	0.030
Look Dam	68.17±6.76	62.17±5.43	0.032	13.67±1.97	19.42±3.88	< 0.001
Koo Ning	67.92±15.04	63.00±5.87	0.324	13.33±3.79	18.33±3.99	0.006
Meuang Sai	65.25±20.64	54.42±19.97	0.224	13.42±5.06	13.17±7.91	0.930
Maw Arun	84.17±16.71	83.50±11.47	0.914	14.92±3.66	17.42±3.50	0.116
Sahn Suay	85.50±25.79	84.50±19.69	0.919	17.58±6.25	19.33±5.86	0.505
Leb Nok	64.58±11.93	62.17±17.14	0.705	10.92±1.89	13.83±4.69	0.069
Sang Yod	79.92±17.71	66.42±10.05	0.039	18.17±5.67	22.66±4.70	0.055
Hawm Bai-Tuey	73.33±24.51	65.25±13.44	0.348	14.58±3.68	16.67±5.14	0.286
Hawm Tammasat	94.92±19.12	90.50±11.40	0.517	14.25±2.74	16.83±3.31	0.059
RD15	102.58±11.69	94.08±10.47	0.086	15.00±2.20	21.33±2.87	< 0.001
Pathumthani 1	109.92±22.58	92.17±10.07	0.026	24.58±6.12	28.50±4.43	0.099
KDML105	116.00±11.93	92.50±11.39	< 0.001	21.25±3.42	21.83±5.18	0.758
Average±SD	82.43±14.64	74.88±11.87	0.088	16.80±4.25	19.15±3.47	0.068
Pup1-K46^{-}						
Look Khuey	77.08±5.51	72.17±11.42	0.212	16.00±1.53	19.83±2.79	0.001
Look Taw	102.75±13.66	77.50±6.56	< 0.001	24.75±3.90	21.08±4.91	0.065
Chana	82.08±9.87	75.42±5.87	0.067	21.00±3.34	19.33±2.66	0.209
Man Tom	86.42±12.82	81.58±10.62	0.346	16.17±3.85	20.17±4.51	0.036
Rai Sai	68.42±11.51	55.08±15.45	0.032	13.83±3.46	12.67±5.96	0.580
Poo Yoo	88.67±22.12	78.58±18.32	0.257	13.50±3.23	16.50±5.25	0.121
Khao Rak	88.75±10.99	83.75±25.45	0.556	17.25±2.31	21.75±8.33	0.098
Nahng Loi	78.00±17.70	74.17±7.90	0.519	17.75±4.85	19.67±3.66	0.307
Puang Wai	88.00±21.68	69.83±11.65	0.023	15.75±3.56	14.42±2.98	0.352
Gan Tang	74.50±20.70	72.08±14.53	0.754	13.08±2.72	17.17±4.24	0.013
Glib Mek	95.12±6.64	85.42±6.49	0.002	17.92±2.06	22.33±2.46	< 0.001
Klah Nak	76.58±28.37	64.42±9.16	0.190	20.83±6.35	23.33±5.79	0.345
Sae Mah	86.58±25.38	69.00±14.36	0.058	15.83±5.57	21.00±8.18	0.097
Tia Malay-Daeng	107.75±12.04	79.58±8.85	< 0.001	21.25±3.39	23.33±2.95	0.139
Khem Thawng	104.00±16.77	89.92±8.69	0.022	19.75±4.80	28.08±4.79	< 0.001
Look Plah	75.50±18.80	54.50±9.50	0.003	10.33±2.43	12.83±3.93	0.087
Malay	108.92±16.37	80.67±13.78	< 0.001	22.67±5.79	21.83±4.43	0.708
RD 41	87.33±13.30	80.00±7.58	0.152	15.58±3.44	20.25±3.65	0.005
Mlea-Hawm	118.08±20.82	100.42±16.73	0.039	16.08±3.12	21.33±4.75	0.006
RD61	100.25±29.14	92.42±16.31	0.445	16.42±4.99	23.08±6.60	0.014
Average±SD	89.74±13.15	76.82±11.05	0.002	17.28±3.45	20.00±3.68	0.024

Table S6 Biomass of the *Pup1*-K46^{+} and *Pup1*-K46^{-} rice cultivars full-strength Yoshida solution (n = 20) (continued)

Cultivars	Total dry weight (mg)			Root/shoot ratio		
	HP	LP	*P*-values	HP	LP	*P*-values
Pup1-K46^{+}						
Kaen Chan	94.25±8.85	100.83±9.25	0.102	0.20±0.03	0.28±0.03	< 0.001
Mae Mai	94.08±8.72	93.92±5.11	0.957	0.21±0.03	0.28±0.03	< 0.001
Hal Ham Doo Lila	78.67±9.24	81.83±6.83	0.371	0.21±0.02	0.26±0.03	< 0.000
Chaw La Mai	118.17±18.60	97.75±6.83	0.003	0.26±0.04	0.21±0.03	0.014
Hua Nadam	95.92±10.33	102.00±7.54	0.129	0.22±0.03	0.30±0.02	< 0.001
Buang Khao	107.83±6.63	94.08±7.18	< 0.001	0.19±0.03	0.21±0.02	0.002
Eaw Mod Daeng	107.83±14.37	92.75±5.46	0.004	0.34±0.05	0.28±0.04	0.002
Lam Hak	83.92±19.24	77.50±11.72	0.355	0.20±0.02	0.28±0.04	< 0.001
Look Dam	81.83±7.88	81.58±8.86	0.945	0.20±0.03	0.31±0.04	< 0.001
Koo Ning	81.25±18.61	81.33±9.39	0.990	0.19±0.02	0.29±0.05	< 0.001
Meuang Sai	78.67±25.59	67.58±27.76	0.341	0.20±0.02	0.23±0.06	0.229
Maw Arun	99.08±20.19	100.92±14.37	0.808	0.18±0.02	0.21±0.03	0.004
Sahn Suay	103.08±31.73	103.83±24.97	0.951	0.20±0.03	0.23±0.04	0.103
Leb Nok	75.50±13.31	76.00±21.68	0.949	0.17±0.03	0.22±0.01	< 0.001
Sang Yod	98.08±22.58	89.08±14.01	0.273	0.23±0.04	0.34±0.05	< 0.001
Hawm Bai-Tuey	87.92±27.62	81.92±18.20	0.554	0.21±0.04	0.25±0.03	0.010
Hawm Tammasat	109.17±21.43	107.33±14.50	0.816	0.15±0.02	0.18±0.02	< 0.001
RD15	117.58±13.47	115.42±12.47	0.699	0.15±0.01	0.23±0.03	< 0.001
Pathumthani 1	134.50±27.17	120.67±14.09	0.148	0.23±0.05	0.31±0.03	< 0.001
KDML105	137.25±14.26	114.33±15.78	0.002	0.18±0.02	0.24±0.04	0.001
Average±SD	99.23±17.47	94.03±14.07	0.319	0.20±0.04	0.26±0.04	< 0.001
Pup1-K46^{-}						
Look Khuey	93.08±6.64	92.00±13.80	0.817	0.21±0.01	0.28±0.02	< 0.001
Look Taw	127.50±17.39	98.58±11.05	< 0.001	0.24±0.01	0.27±0.04	0.047
Chana	103.08±12.34	94.75±6.63	0.061	0.26±0.03	0.26±0.04	0.936
Man Tom	102.58±15.82	101.75±14.60	0.899	0.19±0.03	0.25±0.03	< 0.001
Rai Sai	82.25±14.28	67.75±21.13	0.073	0.20±0.04	0.22±0.05	0.376
Poo Yoo	102.17±25.19	95.08±22.07	0.490	0.15±0.01	0.21±0.05	0.001
Khao Rak	106.00±12.96	105.50±33.34	0.963	0.19±0.02	0.25±0.04	< 0.001
Nahng Loi	95.75±21.64	93.83±11.16	0.796	0.23±0.04	0.26±0.03	0.031
Puang Wai	103.75±25.11	84.25±14.11	0.035	0.18±0.01	0.21±0.02	0.004
Gan Tang	87.58±22.78	89.25±18.30	0.852	0.18±0.03	0.24±0.03	< 0.001
Glib Mek	113.08±8.16	107.75±8.53	0.148	0.19±0.02	0.26±0.02	< 0.001
Klah Nak	97.42±34.15	87.75±14.43	0.396	0.29±0.08	0.36±0.05	0.022
Sae Mah	102.42±29.39	90.00±21.80	0.273	0.19±0.06	0.30±0.07	0.001
Tia Malay-Daeng	129.00±14.04	102.92±10.31	< 0.001	0.20±0.03	0.30±0.04	< 0.001
Khem Thawng	123.75±20.08	118.00±12.58	0.430	0.19±0.03	0.31±0.04	< 0.001
Look Plah	85.83±20.18	67.33±12.99	0.018	0.14±0.03	0.23±0.04	< 0.001
Malay	131.58±19.05	102.50.16.87	0.001	0.21±0.06	0.27±0.04	0.011
RD 41	102.92±16.76	100.25±10.22	0.657	0.18±0.04	0.25±0.04	< 0.001
Mlea-Hawm	134.17±23.26	121.75±20.08	0.194	0.14±0.02	0.21±0.04	< 0.001
RD61	116.67±33.94	115.50±22.70	0.925	0.16±0.01	0.25±0.03	< 0.001
Average±SD	107.03±15.19	96.83±13.82	0.036	0.20±0.04	0.26±0.04	< 0.001

Table S7 P concentration of the *Pup1*-K46^{+} and *Pup1*-K46^{-} rice cultivars in the root and shoot under full-strength Yoshida solution (n = 20)

Cultivars	Total P concentration (mg P/ Kg DW)			
	Shoot		Root	
	HP	LP	HP	LP
Pup1-K46^{+}				
Kaen Chan	5055.33±107.79	732.90±28.12	4056.33±413.42	1048.33±34.57
Mae Mai	5776.33±225.11	740.86±3.67	3816.00±204.56	918.96±42.75
Hal Ham Doo Lila	5032.00±353.56	720.10±60.54	6199.66±583.13	1025.66±65.70
Chaw La Mai	4706.66±788.66	841.30±55.83	4878.67±749.88	942.93±65.18
Hua Nadam	6156.00±202.86	813.26±35.66	6189.66±118.31	1192.33±127.99
Buang Khao	4823.33±63.51	783.56±15.32	4926.66±309.44	1010.36±66.95
Eaw Mod Daeng	4070.33±667.86	705.93±46.21	7017.33±540.99	1102.33±21.64
Lam Hak	5618.00±184.93	784.00±10.94	4752.33±232.37	1028.86±98.89
Look Dam	5352.33±483.61	826.60±31.85	6612.66±622.78	1278.66±31.08
Koo Ning	6072.66±407.75	888.60±76.90	7910.00±314.57	1172.66±52.60
Meuang Sai	6554.00±56.66	960.20±91.77	5008.33±479.29	1202.66±103.51
Maw Arun	7070.00±353.56	910.43±29.58	4515.33±146.35	751.06±35.00
Sahn Suay	6422.33±878.70	863.46±34.98	2677.33±496.77	921.93±86.22
Leb Nok	6884.33±329.58	938.50±56.83	4368.33±496.93	778.20±57.22
Sang Yod	5204.00±377.73	802.60±30.77	5666.00±558.96	1078.90±74.10
Hawm Bai-Tuey	7049.00±815.33	1218.00±63.89	8595.33±113.04	1416.66±61.56
Hawm Tammasat	6701.66±83.11	977.56±25.80	4897.33±500.31	1014.40±125.77
RD15	6015.00±132.23	962.33±33.78	5040.33±657.98	866.90±38.66
Pathumthani 1	5579.00±254.95	1002.63±41.98	5899.33±983.25	914.53±46.91
KDML105	6561.66±202.17	951.10±48.99	4098.00±292.76	1312.33±125.32
Average±SD	5835.20±936.09	871.20±129.11	5356.25±1478.61	1048.94±186.46
P-value	< 0.001		< 0.001	
Pup1-K46^{-}				
Look Khuey	6950.00±292.16	811.13±32.41	3669.33±489.49	765.90±33.34
Look Taw	5036.33±381.98	686.53±53.97	4240.33±433.61	706.10±79.02
Chana	5743.00±155.37	898.40±29.36	3866.33±450.27	792.63±110.41
Man Tom	5175.00±304.18	838.73±26.62	4286.33±256.51	1019.40±89.90
Rai Sai	6371.66±99.57	907.20±105.98	4106.00±526.52	1163.33±54.93
Poo Yoo	5973.00±339.44	978.43±47.26	3265.00±324.97	717.36±26.62
Khao Rak	6548.33±347.24	1029.60±34.01	4819.33±769.54	697.90±49.43
Nahng Loi	4881.66±137.89	704.66±35.59	4205.66±494.67	875.90±44.01
Puang Wai	5145.00±297.40	770.20±22.93	3229.66±511.26	773.10±103.88
Gan Tang	5900.33±375.77	868.16±96.70	4448.33±668.29	826.06±90.91
Glib Mek	6928.00±223.57	794.76±20.09	3754.66±564.90	740.33±72.14
Klah Nak	5278.00±106.10	783.36±54.66	6099.66±497.87	909.10±76.05
Sae Mah	5055.00±131.61	781.63±35.27	5525.33±517.16	1042.33±37.81
Tia Malay-Daeng	4693.00±542.76	711.26±56.55	5285.66±522.52	826.56±52.97
Khem Thawng	4624.66±539.40	752.13±32.31	5110.00±944.11	1081.90±109.63
Look Plah	5873.33±273.77	776.96±77.12	2801.33±240.31	1253.66±37.75
Malay	4561.66±251.34	719.60±38.40	4761.33±625.71	808.43±83.56
RD 41	5174.00±241.15	893.16±41.92	4220.00±908.47	847.93±70.68
Mlea-Hawm	5390.00±148.60	872.70±23.67	3138.00±73.61	659.93±63.01
RD61	5497.33±424.28	924.10±89.13	4254.66±519.74	867.70±118.60
Average±SD	5539.97±772.93	825.14±106.59	4254.35±993.95	868.78±176.10
P-value	< 0.001		< 0.001	

Table S8 P uptake of the *Pup1*-K46^{+} and *Pup1*-K46^{-} cultivars in the shoot and root under full-strength Yoshida solution (n = 20)

Cultivars	P Uptake (mg P/ plant)			
	Shoot		Root	
	HP	LP	HP	LP
Pup1-K46^{+}				
Kaen Chan	0.40	0.06	0.06	0.02
Mae Mai	0.45	0.05	0.06	0.02
Hal Ham Doo Lila	0.33	0.05	0.08	0.02
Chaw La Mai	0.44	0.07	0.12	0.02
Hua Nadam	0.48	0.06	0.11	0.03
Buang Khao	0.44	0.06	0.08	0.02
Eaw Mod Daeng	0.33	0.05	0.19	0.02
Lam Hak	0.39	0.05	0.06	0.02
Look Dam	0.36	0.05	0.09	0.02
Koo Ning	0.41	0.06	0.11	0.02
Meuang Sai	0.43	0.05	0.07	0.02
Maw Arun	0.60	0.08	0.07	0.01
Sahn Suay	0.55	0.07	0.05	0.02
Leb Nok	0.44	0.06	0.05	0.01
Sang Yod	0.42	0.05	0.10	0.02
Hawm Bai-Tuey	0.52	0.08	0.13	0.02
Hawm Tammasat	0.64	0.09	0.07	0.02
RD15	0.62	0.09	0.08	0.02
Pathumthani 1	0.61	0.09	0.15	0.03
KDML105	0.76	0.09	0.09	0.03
Average±SD	0.48±0.11	0.07±0.02	0.09±0.03	0.020±0.00
P-value	< 0.001		< 0.001	
Pup1-K46^{-}				
Look Khuey	0.54	0.06	0.06	0.02
Look Taw	0.52	0.05	0.10	0.01
Chana	0.47	0.07	0.08	0.02
Man Tom	0.45	0.07	0.07	0.02
Rai Sai	0.44	0.05	0.06	0.01
Poo Yoo	0.53	0.08	0.04	0.01
Khao Rak	0.58	0.09	0.08	0.02
Nahng Loi	0.38	0.05	0.07	0.02
Puang Wai	0.45	0.05	0.05	0.01
Gan Tang	0.44	0.06	0.06	0.01
Glib Mek	0.66	0.07	0.07	0.02
Klah Nak	0.40	0.05	0.13	0.02
Sae Mah	0.44	0.05	0.09	0.02
Tia Malay-Daeng	0.51	0.06	0.11	0.02
Khem Thawng	0.48	0.07	0.10	0.03
Look Plah	0.44	0.04	0.03	0.02
Malay	0.50	0.06	0.11	0.02
RD 41	0.45	0.07	0.07	0.02
Mlea-Hawm	0.64	0.09	0.05	0.01
RD61	0.55	0.09	0.07	0.02
Average±SD	0.50±0.07	0.06±0.01	0.07±0.02	0.017±0.00
P-value	< 0.001		< 0.001	

Table S9 PUE of the *Pup1*-K46^{+} and *Pup1*-K46^{-} cultivars in the shoot and root under full-strength Yoshida solution (n = 20)

Cultivars	PUE (g DW/mg P)			
	Shoot		Root	
	HP	LP	HP	LP
Pup1-K46^{+}				
Kaen Chan	0.20±0.00	1.37±0.05	0.25±0.02	0.95±0.03
Mae Mai	0.17±0.01	1.35±0.01	0.26±0.01	1.09±0.05
Hal Ham Doo Lila	0.20±0.01	1.30±0.12	0.16±0.02	0.98±0.06
Chaw La Mai	0.22±0.03	1.19±0.08	0.21±0.03	1.07±0.07
Hua Nadam	0.16±0.01	1.23±0.05	0.16±0.00	0.85±0.10
Buang Khao	0.21±0.00	1.28±0.02	0.20±0.01	0.99±0.07
Eaw Mod Daeng	0.25±0.04	1.42±0.09	0.14±0.01	0.91±0.02
Lam Hak	0.18±0.01	1.28±0.02	0.21±0.01	0.98±0.09
Look Dam	0.19±0.02	1.21±0.05	0.15±0.01	0.78±0.02
Koo Ning	0.17±0.01	1.13±0.10	0.13±0.00	0.85±0.04
Meuang Sai	0.15±0.00	1.05±0.09	0.20±0.02	0.84±0.08
Maw Arun	0.14±0.01	1.10±0.04	0.22±0.01	1.33±0.06
Sahn Suay	0.16±0.02	1.16±0.05	0.39±0.08	1.10±0.10
Leb Nok	0.15±0.01	1.07±0.06	0.23±0.03	1.29±0.10
Sang Yod	0.19±0.01	1.25±0.05	0.18±0.02	0.93±0.07
Hawm Bai-Tuey	0.14±0.02	0.82±0.04	0.12±0.00	0.70±0.03
Hawm Tammasat	0.15±0.00	1.02±0.03	0.21±0.02	1.00±0.12
RD15	0.17±0.00	1.04±0.04	0.20±0.02	1.16±0.05
Pathumthani 1	0.18±0.01	1.00±0.04	0.18±0.03	1.10±0.06
KDML105	0.15±0.00	1.05±0.05	0.25±0.02	0.77±0.07
Average±SD	0.18±0.03	1.17±0.15	0.20±0.06	0.98±0.16
P-value	< 0.001		< 0.001	
Pup1-K46^{-}				
Look Khuey	0.14±0.01	1.23±0.05	0.28±0.04	1.31±0.06
Look Taw	0.20±0.02	1.47±0.11	0.24±0.03	1.43±0.17
Chana	0.17±0.00	1.11±0.04	0.26±0.03	1.28±0.16
Man Tom	0.19±0.01	1.19±0.04	0.23±0.01	0.99±0.09
Rai Sai	0.16±0.00	1.12±0.13	0.25±0.03	0.86±0.04
Poo Yoo	0.17±0.01	1.02±0.05	0.31±0.03	1.34±0.05
Khao Rak	0.15±0.01	0.97±0.03	0.21±0.03	1.44±0.10
Nahng Loi	0.21±0.01	1.42±0.07	0.24±0.03	1.14±0.06
Puang Wai	0.20±0.01	1.30±0.04	0.32±0.06	1.32±0.06
Gan Tang	0.17±0.01	1.17±0.13	0.23±0.04	1.23±0.13
Glib Mek	0.14±0.00	1.26±0.03	0.27±0.04	1.36±0.13
Klah Nak	0.19±0.00	1.28±0.09	0.17±0.01	1.11±0.09
Sae Mah	0.20±0.01	1.28±0.06	0.18±0.02	0.96±0.03
Tia Malay-Daeng	0.22±0.03	1.41±0.11	0.19±0.02	1.21±0.08
Khem Thawng	0.22±0.02	1.33±0.06	0.20±0.04	0.93±0.10
Look Plah	0.17±0.01	1.30±0.12	0.36±0.03	0.80±0.02
Malay	0.22±0.01	1.39±0.08	0.21±0.03	1.25±0.14
RD 41	0.19±0.01	1.12±0.05	0.25±0.06	1.19±0.11
Mlea-Hawm	0.19±0.01	1.15±0.03	0.32±0.01	1.53±0.15
RD61	0.18±0.01	1.09±0.10	0.24±0.03	1.17±0.15
Average±SD	0.18±0.02	1.23±0.13	0.25±0.05	1.20±0.20
P-value	< 0.001		< 0.001	

Table S10 Pi content of the *Pup1*-K46^{+} and *Pup1*-K46^{-} cultivars under the HP, LP and PR in the shoot and root at full-strength Yoshida solution (n = 20)

Cultivars	Phosphate (nmol P/ mg FW)					
	Shoot			Root		
	HP	LP	PR	HP	LP	PR
Pup1-K46^{+}						
Kaen Chan	36.29±2.63	1.54±0.10	15.42±1.00	4.08±1.18	1.36±0.14	7.04±0.50
Mae Mai	38.30±2.67	2.45±0.39	18.76±2.23	6.22±0.51	1.55±0.10	7.98±0.83
Hal Ham Doo Lila	35.96±2.49	2.23±0.11	16.88±2.47	8.14±0.93	3.09±0.12	8.29±0.64
Chaw La Mai	35.20±2.00	2.25±0.58	12.54±2.51	4.90±0.50	2.21±0.24	8.65±1.14
Hua Nadam	35.36±2.41	2.28±0.25	12.49±1.05	5.40±0.05	2.03±0.27	8.27±1.16
Buang Khao	31.88±0.75	1.68±0.09	14.00±1.62	5.28±0.54	1.82±0.20	9.49±0.71
Eaw Mod Daeng	32.42±1.74	1.41±0.16	12.42±1.36	4.71±0.32	1.62±020	7.17±0.87
Lam Hak	33.67±0.54	1.73±0.19	14.66±0.97	5.47±0.09	1.84±0.28	9.50±0.73
Look Dam	32.83±0.79	2.13±0.21	6.59±1.08	4.72±0.34	1.82±0.33	2.83±0.43
Koo Ning	35.79±1.81	2.71±0.53	21.71±1.11	6.18±0.27	2.20±0.19	9.45±1.30
Meuang Sai	35.61±1.42	2.39±0.25	17.48±1.02	6.34±0.15	3.32±0.82	8.39±0.36
Maw Arun	37.83±2.22	2.56±0.24	17.54±0.89	5.94±0.97	1.94±0.28	9.26±1.17
Sahn Suay	36.29±1.24	2.09±0.21	15.98±1.26	4.24±0.22	1.33±0.05	5.11±0.59
Leb Nok	37.44±1.62	2.16±0.21	13.28±3.16	5.85±0.75	2.08±0.17	7.38±0.71
Sang Yod	33.29±1.08	1.79±0.04	14.08±1.74	4.83±0.57	1.64±0.10	7.58±0.58
Hawm Bai-Tuey	35.07±2.24	2.82±0.28	17.78±1.09	5.10±0.26	1.63±0.09	6.83±0.39
Hawm Tammasat	34.21±1.92	2.37±0.43	14.60±0.50	5.88±0.48	1.40±0.31	4.78±0.37
RD15	31.70±2.60	1.99±0.23	15.36±2.35	5.32±0.74	1.42±0.31	4.65±0.90
Pathumthani 1	33.49±1.73	1.82±0.16	11.54±0.73	6.84±0.67	1.26±0.11	3.13±0.69
KDML105	32.75±0.87	1.7±0.22	13.45±1.05	4.56±0.13	1.04±0.24	3.44±0.13
Average±SD	34.77±2.67	2.11±0.47	14.98±2.97	5.50±1.10	1.83±0.62	6.90±2.11
Pup1-K46^{-}						
Look Khuey	34.53±1.14	2.14±0.18	14.50±1.99	5.64±0.65	1.09±0.12	8.54±0.89
Look Taw	30.57±1.05	1.33±0.10	12.81±1.54	4.75±0.17	0.91±0.11	7.10±0.41
Chana	34.36±1.89	1.48±0.16	8.50±1.43	3.66±0.20	1.31±0.22	3.06±0.19
Man Tom	33.34±3.37	1.57±0.39	18.28±1.08	4.25±0.52	1.08±0.15	6.06±0.56
Rai Sai	33.75±1.13	1.90±0.43	18.91±1.91	4.88±0.42	1.39±0.18	8.01±0.86
Poo Yoo	33.52±0.69	2.38±0.12	19.48±1.37	5.16±0.46	1.90±0.15	9.89±0.44
Khao Rak	34.32±0.86	1.77±0.12	16.40±1.29	6.68±0.78	1.93±0.16	10.48±0.86
Nahng Loi	32.45±1.57	1.38±0.18	11.42±0.63	3.58±0.27	1.62±0.19	6.56±1.49
Puang Wai	35.11±1.19	1.40±0.18	14.15±1.75	4.95±0.54	1.67±0.35	8.16±0.96
Gan Tang	31.28±0.74	1.47±0.27	16.28±2.18	5.83±1.13	1.97±0.39	11.62±1.06
Glib Mek	33.59±1.30	1.75±0.37	15.13±1.71	6.18±0.64	1.52±0.24	7.11±0.85
Klah Nak	35.31±0.66	1.85±0.42	16.31±2.01	5.98±0.37	1.35±0.09	7.16±0.89
Sae Mah	35.34±1.19	1.69±0.27	17.86±1.80	5.12±0.40	1.12±0.21	8.44±0.84
Tia Malay-Daeng	32.43±0.82	1.22±0.21	18.10±2.57	6.27±0.58	0.88±0.08	8.14±1.00
Khem Thawng	36.56±1.48	1.58±0.13	19.29±1.13	6.27±0.69	1.40±0.23	10.37±1.86
Look Plah	33.38±2.39	1.12±0.16	20.78±1.39	5.80±0.98	1.36±0.32	11.69±0.51
Malay	33.28±2.88	1.11±0.15	11.78±2.30	5.85±0.37	1.27±0.09	6.79±2.62
RD 41	32.92±1.97	0.94±0.12	14.13±3.88	5.58±0.95	1.36±0.11	9.01±0.63
Mlea-Hawm	35.22±1.44	1.25±0.11	18.79±2.00	5.05±0.30	1.08±0.16	7.47±1.36
RD61	34.18±1.42	0.97±0.31	16.76±1.34	5.52±0.90	1.40±0.43	8.58±1.53
Average±SD	33.77±2.14	1.52±0.44	15.98±3.62	5.35±1.03	1.38±0.38	8.21±2.27

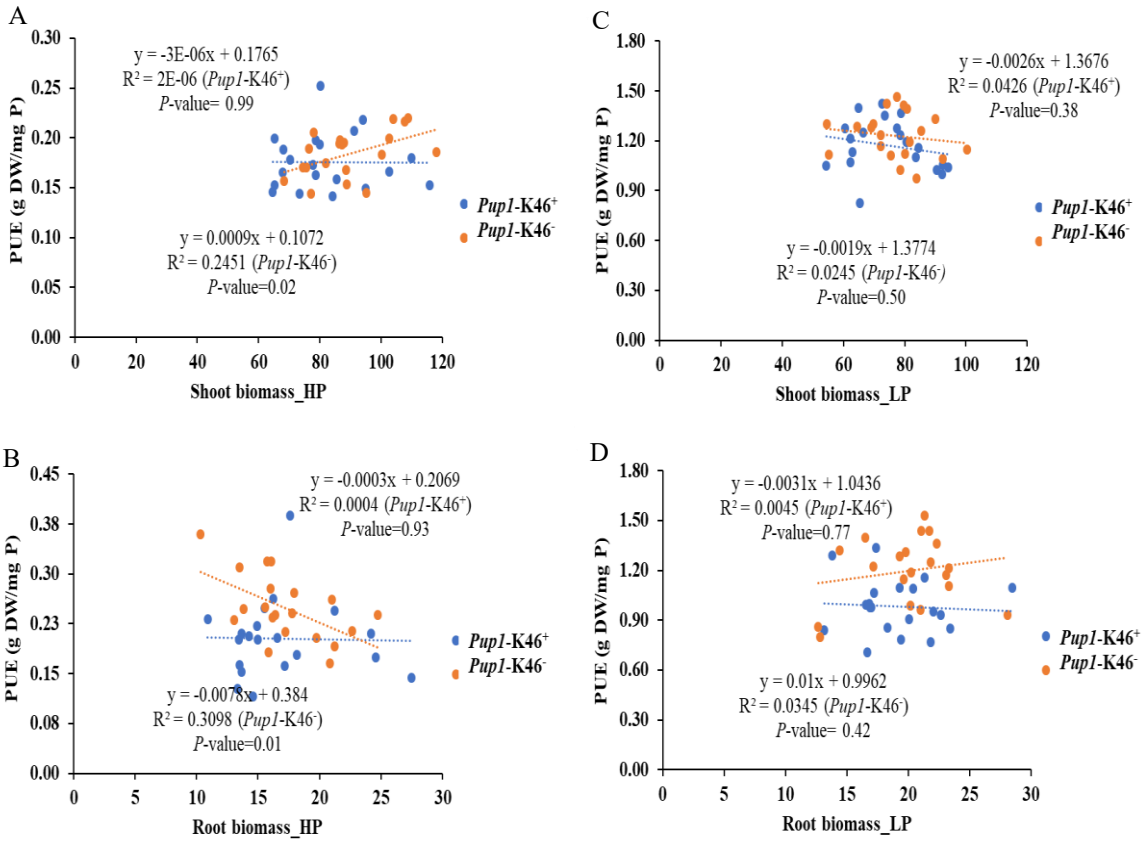


Figure S1 Correlation between PUE and biomass of the shoot and root in the *Pup1*-K46^{+} and *Pup1*-K46^{-} cultivars under the HP and LP condition at full-strength Yoshida solution (n = 20). The correlation was calculated by R software with LSD, (*P*-value ≤ 0.05).

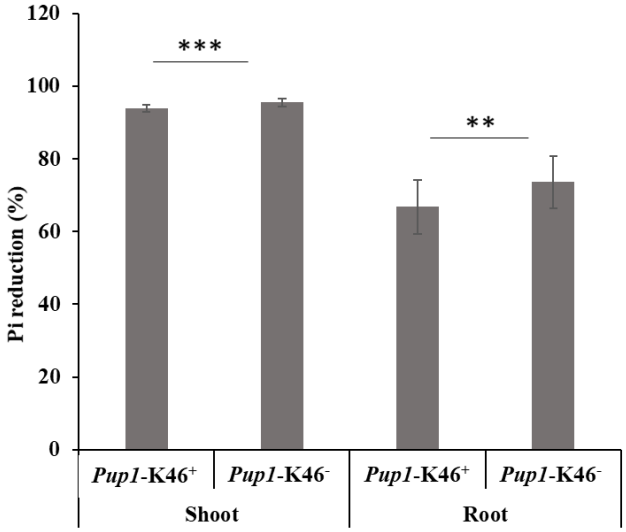


Figure S2 The Pi reduction of the *Pup1*-K46^{+} and *Pup1*-K46^{-} cultivars in the shoot and root under full-strength Yoshida solution (n = 20). Error bars represent SD. Statistical analysis was conducted with student's t-test *, **, and *** indicate significant differences at $P \leq 0.05$, 0.01, and 0.001, respectively.

Appendix D

Data analysis

Chlorophyll content measured by the chlorophyll meter

```
            Df Sum Sq Mean Sq F value   Pr(>F)
Treatment    3   58.9  19.632   11.01 4.42e-06 ***
Residuals   76  135.6   1.784
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
$`statistics`
   MSerror Df   Mean       CV  t.value       LSD
  1.783707 76 23.467 5.691204 1.991673 0.8411621
$parameters
        test p.ajusted    name.t ntr alpha
  Fisher-LSD      none Treatment   4  0.05

$means
             Chl     std  r     LCL     UCL   Min   Max    Q25   Q50    Q75
HP_Pup1-K46- 22.3110 0.9891 20 21.71621 22.9057 19.92 23.85 22.050 22.505 22.7925
HP_Pup1-K46+ 23.1700 1.4770 20 22.57521 23.7647 20.86 25.66 21.825 23.180 24.3375
LP_Pup1-K46- 23.7115 1.2723 20 23.11671 24.3062 20.00 26.02 23.257 23.865 24.4825
LP_Pup1-K46+ 24.6755 1.5348 20 24.08071 25.2702 22.41 27.91 23.280 24.770 25.6300

$comparison
NULL

$groups
         Chlorophyll groups
LP_Pup1-K46+  24.6755      a
LP_Pup1-K46-  23.7115      b
HP_Pup1-K46+  23.1700      b
HP_Pup1-K46-  22.3110      c
```

Dry weight measurement under half-strenght solution

```
             Df Sum Sq Mean Sq F value Pr(>F)
Sample       23 117369    5103    71.8 <2e-16 ***
Residuals   468  33262      71
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
$`statistics`
   MSerror  Df     Mean       CV
  71.07364 468 27.02846 31.19127
$parameters
        test p.ajusted name.t ntr alpha
  Fisher-LSD      none Sample  24  0.05

$means
                Dw       std  r       LCL      UCL   Min Max  Q25  Q50   Q75
R.HP.ML   10.318182  3.014036 22  6.786223 13.85014   5  16  8.00 10.0 12.75
R.HP.TU01 12.157895  2.774150 19  8.357312 15.95848   7  16 11.00 12.0 14.00
R.HP.PT1  16.000000  5.206833 19 12.199417 19.80058   9  26 12.00 16.0 18.00
R.HP.RD61 12.000000  2.981424 19  8.199417 15.80058   8  17  9.50 12.0 14.50
R.HP.BK   11.227273  1.659943 22  7.695314 14.75923   8  13 10.00 11.0 13.00
R.HP.KT   11.500000  5.289252 22  7.968041 15.03196   3  24  8.50 12.0 14.00
R.LP.ML   10.090909  2.958589 22  6.558950 13.62287   5  16  8.00 11.0 12.00
R.LP.TU01 12.263158  3.124137 19  8.462575 16.06374   7  18 10.00 12.0 14.50
R.LP.PT1  14.052632  3.978745 19 10.252049 17.85321   8  21 11.00 15.0 15.50
R.LP.RD61 11.473684  2.931977 19  7.673102 15.27427   7  18  9.00 11.0 13.50
R.LP.BK   11.818182  1.562549 22  8.286223 15.35014  10  15 11.00 11.5 13.00
R.LP.KT    9.863636  4.443245 22  6.331678 13.39560   3  21  7.00 10.0 13.00
S.HP.ML   42.500000 10.432322 22 38.968041 46.03196  24  62 33.50 45.0 48.00
S.HP.TU01 44.473684 11.936724 19 40.673102 48.27427  17  63 40.00 44.0 52.50
S.HP.PT1  39.105263 11.911221 19 35.304681 42.90585  16  60 30.50 41.0 48.00
S.HP.RD61 44.263158  9.409464 19 40.462575 48.06374  30  61 36.50 44.0 51.00
S.HP.BK   46.636364  4.981351 22 43.104405 50.16832  36  54 43.25 48.0 50.50
S.HP.KT   47.227273 20.332783 22 43.695314 50.75923  10  83 31.75 49.5 60.00
S.LP.ML   37.181818  8.174504 22 33.649859 40.71378  22  53 30.50 39.0 42.50
S.LP.TU01 43.368421  9.423437 19 39.567838 47.16900  22  56 35.50 45.0 51.00
S.LP.PT1  38.842105  9.771064 19 35.041523 42.64269  21  59 33.50 41.0 43.00
S.LP.RD61 40.736842  8.581907 19 36.936260 44.53742  28  57 33.00 41.0 47.50
S.LP.BK   44.909091  4.471168 22 41.377132 48.44105  35  53 42.00 45.0 47.75
S.LP.KT   37.272727 16.487172 22 33.740768 40.80469  15  71 27.00 34.0 46.50

$comparison
NULL
$groups
                Dw  groups                        Dw  groups
S.HP.KT   47.227273       a          R.LP.TU01 12.263158      fg
S.HP.BK   46.636364       a          R.HP.TU01 12.157895      fg
S.LP.BK   44.909091      ab          R.HP.RD61 12.000000      fg
S.HP.TU01 44.473684     abc          R.LP.BK   11.818182      fg
S.HP.RD61 44.263158     abc          R.HP.KT   11.500000      fg
S.LP.TU01 43.368421    abcd          R.LP.RD61 11.473684      fg
S.HP.ML   42.500000    abcd          R.HP.BK   11.227273      fg
S.LP.RD61 40.736842    bcde          R.HP.ML   10.318182       g
S.HP.PT1  39.105263     cde          R.LP.ML   10.090909       g
S.LP.PT1  38.842105      de          R.LP.KT    9.863636       g
S.LP.KT   37.272727       e
S.LP.ML   37.181818       e
R.HP.PT1  16.000000       f
R.LP.PT1  14.052632      fg
```

Total P concentration measurement under half-strength solution

```
                Df    Sum Sq Mean Sq F value Pr(>F)
Sample      23 170899386 7430408   91.07 <2e-16 ***
Residuals   48   3916494   81594
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
$`statistics`
   MSerror Df     Mean       CV  t.value      LSD
  81593.62 48 2263.343 12.62053 2.010635 468.9382
$parameters
        test p.ajusted name.t ntr alpha
  Fisher-LSD      none Sample  24  0.05
$means
                TP        std r       LCL      UCL    Min    Max     Q25    Q50    Q75
R.HP.ML   1857.6667 168.09025 3 1526.0773 2189.256 1726.0 2047.0 1763.00 1800.0 1923.5
R.HP.TU01 1947.3333 413.83129 3 1615.7439 2278.923 1697.0 2425.0 1708.50 1720.0 2072.5
R.HP.PT1  1994.6667 138.85724 3 1663.0773 2326.256 1836.0 2094.0 1945.00 2054.0 2074.0
R.HP.RD61 2585.3333 387.44978 3 2253.7439 2916.923 2180.0 2952.0 2402.00 2624.0 2788.0
R.HP.BK   1352.3333 162.52487 3 1020.7439 1683.923 1258.0 1540.0 1258.50 1259.0 1399.5
R.HP.KT   2627.0000 222.60279 3 2295.4106 2958.589 2479.0 2883.0 2499.00 2519.0 2701.0
R.LP.ML   800.6000   35.91490 3  469.0106 1132.189  777.8  842.0  779.90  782.0  812.0
R.LP.TU01 957.3667  190.36623 3  625.7773 1288.956  747.1 1118.0 877.05  1007.0 1062.5
R.LP.PT1  1139.3333  78.37304 3  807.7439 1470.923 1053.0 1206.0 1106.00 1159.0 1182.5
R.LP.RD61 1116.5333 181.52205 3  784.9439 1448.123  916.6 1271.0 1039.30 1162.0 1216.5
R.LP.BK   751.4333  108.56230 3  419.8439 1083.023  629.1  836.3  709.00  788.9  812.6
R.LP.KT   1193.6667 185.76957 3  862.0773 1525.256 1079.0 1408.0 1086.50 1094.0 1251.0
S.HP.ML   4250.6667 152.51994 3 3919.0773 4582.256 4137.0 4424.0 4164.00 4191.0 4307.5
S.HP.TU01 5082.6667 424.11359 3 4751.0773 5414.256 4646.0 5493.0 4877.50 5109.0 5301.0
S.HP.PT1  4046.6667 280.48945 3 3715.0773 4378.256 3819.0 4360.0 3890.00 3961.0 4160.5
S.HP.RD61 4914.6667  94.39456 3 4583.0773 5246.256 4831.0 5017.0 4863.50 4896.0 4956.5
S.HP.BK   4348.0000 209.22954 3 4016.4106 4679.589 4156.0 4571.0 4236.50 4317.0 4444.0
S.HP.KT   5856.6667 950.21278 3 5525.0773 6188.256 4825.0 6696.0 5437.00 6049.0 6372.5
S.LP.ML   1247.0000 149.29166 3  915.4106 1578.589 1151.0 1419.0 1161.00 1171.0 1295.0
S.LP.TU01 1182.3333  94.87009 3  850.7439 1513.923 1116.0 1291.0 1128.00 1140.0 1215.5
S.LP.PT1  1175.0000  42.50882 3  843.4106 1506.589 1133.0 1218.0 1153.50 1174.0 1196.0
S.LP.RD61 1600.3333 183.50568 3 1268.7439 1931.923 1398.0 1756.0 1522.50 1647.0 1701.5
S.LP.BK   1036.4667  67.36953 3  704.8773 1368.056  967.4 1102.0 1003.70 1040.0 1071.0
S.LP.KT   1256.5000 285.90951 3  924.9106 1588.089  952.5 1520.0 1124.75 1297.0 1408.5

$comparison
NULL
$groups
                TP groups                         TP groups
S.HP.KT   5856.6667      a         S.LP.TU01 1182.3333   fghi
S.HP.TU01 5082.6667      b         S.LP.PT1  1175.0000   fghi
S.HP.RD61 4914.6667      b         R.LP.PT1  1139.3333   fghi
S.HP.BK   4348.0000      c         R.LP.RD61 1116.5333    ghi
S.HP.ML   4250.6667      c         S.LP.BK   1036.4667    ghi
S.HP.PT1  4046.6667      c         R.LP.TU01  957.3667    ghi
R.HP.KT   2627.0000      d         R.LP.ML    800.6000     hi
R.HP.RD61 2585.3333      d         R.LP.BK    751.4333      i
R.HP.PT1  1994.6667      e
R.HP.TU01 1947.3333      e
R.HP.ML   1857.6667      e
S.LP.RD61 1600.3333     ef
R.HP.BK   1352.3333     fg
S.LP.KT   1256.5000    fgh
S.LP.ML   1247.0000    fgh
R.LP.KT   1193.6667   fghi
```

PUE measurement under half-strength solution

```
            Df Sum Sq Mean Sq F value Pr(>F)
Sample      23  8.307  0.3612   36.74 <2e-16 ***
Residuals   48  0.472  0.0098
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
$`statistics`
      MSerror Df      Mean       CV  t.value       LSD
  0.009831211 48 0.6515662 15.21756 2.010635 0.1627763

$parameters
        test p.ajusted name.t ntr alpha
  Fisher-LSD      none Sample  24  0.05
$means
             PUE        std r      LCL      UCL      Min      Max       Q25      Q50      Q75
R.HP.ML   0.541149 0.04710920 3 0.4260496 0.656250 0.488519 0.579374 0.5220377 0.5555556 0.5674649
R.HP.TU01 0.527680 0.09993858 3 0.4125803 0.642780 0.412371 0.589275 0.4968832 0.5813953 0.5853353
R.HP.PT1  0.503024 0.03635837 3 0.3879238 0.618124 0.477554 0.544662 0.4822049 0.4868549 0.5157586
R.HP.RD61 0.392855 0.06083929 3 0.2777553 0.507955 0.338753 0.458715 0.3599255 0.3810976 0.4199066
R.HP.BK   0.746181 0.08385853 3 0.6310812 0.861281 0.649350 0.794912 0.7218159 0.7942812 0.7945969
R.HP.KT   0.382410 0.03095322 3 0.2673105 0.497511 0.346860 0.403388 0.3719219 0.3969829 0.4001857
R.LP.ML   1.250699 0.05471281 3 1.1355992 1.365799 1.187648 1.285677 1.2332104 1.2787724 1.2822250
R.LP.TU01 1.075337 0.23318377 3 0.9602371 1.190437 0.894454 1.338508 0.9437515 0.9930487 1.1657788
R.LP.PT1  0.880555 0.06216900 3 0.7654557 0.995656 0.829187 0.949667 0.8460001 0.8628128 0.9062402
R.LP.RD61 0.912785 0.15867895 3 0.7976850 1.027885 0.786782 1.090988 0.8236836 0.8605852 0.9757868
R.LP.BK   1.350967 0.20973683 3 1.2358675 1.466068 1.195743 1.589572 1.2316655 1.2675878 1.4285801
R.LP.KT   0.850362 0.12152704 3 0.7352625 0.965462 0.710227 0.926784 0.8121520 0.9140768 0.9204304
S.HP.ML   0.235455 0.00830186 3 0.1203555 0.350556 0.226039 0.241721 0.2323232 0.2386065 0.2401638
S.HP.TU01 0.197673 0.01667942 3 0.0825737 0.312774 0.182049 0.215238 0.1888915 0.1957330 0.2054860
S.HP.PT1  0.247889 0.01672102 3 0.1327891 0.3629895 0.22935 0.261848 0.2409096 0.2524615 0.2571551
S.HP.RD61 0.203522 0.00388825 3 0.0884221 0.3186226 0.19932 0.206996 0.2017853 0.2042484 0.2056224
S.HP.BK   0.230342 0.01098054 3 0.1152427 0.345443 0.218770 0.240616 0.2252064 0.2316423 0.2361292
S.HP.KT   0.173971 0.02990980 3 0.0588709 0.289071 0.149342 0.207253 0.1573297 0.1653166 0.1862852
S.LP.ML   0.809167 0.09075650 3 0.6940672 0.924267 0.704721 0.868809 0.7793463 0.8539710 0.8613903
S.LP.TU01 0.849281 0.06536571 3 0.7341810 0.964381 0.774593 0.896057 0.8258932 0.8771930 0.8866252
S.LP.PT1  0.851806 0.03079723 3 0.7367062 0.966906 0.8210181 0.88261 0.8364034 0.8517888 0.8672006
S.LP.RD61 0.630649 0.07569914 3 0.5155491 0.745749 0.569476  0.71530 0.5883203 0.6071645 0.6612361
S.LP.BK   0.967559 0.06334375 3 0.8524591 1.0826595 0.907441 1.033698 0.934489 0.9615385 0.9976185
S.LP.KT   0.826257 0.20174278 3 0.7111576 0.9413580 0.657894 1.049868 0.714452 0.7710100 0.9104394

$comparison
NULL
$groups
               PUE groups                          PUE groups
R.LP.BK   1.3509678      a          R.HP.ML   0.5411499     fg
R.LP.ML   1.2506995      a          R.HP.TU01 0.5276806     fg
R.LP.TU01 1.0753373      b          R.HP.PT1  0.5030240     fg
S.LP.BK   0.9675594     bc          R.HP.RD61 0.3928555     gh
R.LP.RD61 0.9127852     bc          R.HP.KT   0.3824108     gh
R.LP.PT1  0.8805559     cd          S.HP.PT1  0.2478893     hi
S.LP.PT1  0.8518065     cd          S.HP.ML   0.2354558     hi
R.LP.KT   0.8503627     cd          S.HP.BK   0.2303429     hi
S.LP.TU01 0.8492812     cd          S.HP.RD61 0.2035224      i
S.LP.KT   0.8262578     cd          S.HP.TU01 0.1976739      i
S.LP.ML   0.8091674     cd          S.HP.KT   0.1739711      i
R.HP.BK   0.7461815     de
S.LP.RD61 0.6306494     ef
```

The correlation between the PUE and P uptake

- **Shoot of the *Pup1*-K46^{+} cultivars under the HP condition**

```
        Pearson's product-moment correlation

data:  Experiment_3_2_$`uptake_HP+` and Experiment_3_2_$`PUE_HP+`
t = -3.1766, df = 18, p-value = 0.005225
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 -0.8234648 -0.2134320
sample estimates:
       cor
-0.5993457
```

- **Shoot of the *Pup1*-K46^{+} cultivars under the LP condition**

```
        Pearson's product-moment correlation

data:  Experiment_3_2_$`uptake_LP+` and Experiment_3_2_$`PUE_LP+`
t = -4.3129, df = 18, p-value = 0.000419
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 -0.8783255 -0.3949667
sample estimates:
       cor
-0.7128882
```

- **Shoot of the *Pup1*-K46^{-} cultivars under the HP condition**

```
        Pearson's product-moment correlation

data:  Experiment_3_2_$`uptake_HP-` and Experiment_3_2_$`PUE_HP-`
t = -1.8002, df = 18, p-value = 0.08862
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 -0.71033716  0.06277543
sample estimates:
       cor
-0.3905959
```

- **Shoot of the *Pup1*-K46^{-} cultivars under the LP condition**

```
        Pearson's product-moment correlation

data:  Experiment_3_2_$`uptake_LP-` and Experiment_3_2_$`PUE_LP-`
t = -1.9768, df = 18, p-value = 0.06358
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 -0.7286812  0.0248074
sample estimates:
       cor
-0.4223499
```

- **Root of the *Pup1*-K46^{+} cultivars under the HP condition**

```
        Pearson's product-moment correlation

data:  Experiment_3_2_$`uptake_HP+` and Experiment_3_2_$`PUE_HP+`
t = -3.4653, df = 18, p-value = 0.002762
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 -0.8399709 -0.2639527
sample estimates:
       cor
-0.6325847
```

- **Root of the *Pup1*-K46^{+} cultivars under the LP condition**

```
        Pearson's product-moment correlation

data:  Experiment_3_2_$`uptake_LP+` and Experiment_3_2_$`PUE_LP+`
t = -4.0309, df = 18, p-value = 0.000784
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 -0.8670315 -0.3542315
sample estimates:
       cor
-0.6887823
```

- **Root of the *Pup1*-K46^{-} cultivars under the HP condition**

```
        Pearson's product-moment correlation

data:  Experiment_3_2_$`uptake_HP-` and Experiment_3_2_$`PUE_HP-`
t = -7.0822, df = 18, p-value = 1.328e-06
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 -0.9425596 -0.6694716
sample estimates:
       cor
-0.8578499
```

- **Root of the *Pup1*-K46^{-} cultivars under the LP condition**

```
        Pearson's product-moment correlation

data:  Experiment_3_2_$`uptake_LP-` and Experiment_3_2_$`PUE_LP-`
t = -2.7059, df = 18, p-value = 0.01447
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 -0.7918317 -0.1249450
sample estimates:
       cor
-0.5377341
```

The correlation between the PUE and biomass

- **Shoot of the *Pup1*-K46^{+} cultivars under the HP condition**

```
        Pearson's product-moment correlation

data:  Experiment_3_2_$`S_HP+` and Experiment_3_2_$`PUE_HP+`
t = -0.0056955, df = 18, p-value = 0.9955
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 -0.4435997  0.4414406
sample estimates:
        cor
-0.00134244
```

- **Shoot of the *Pup1*-K46^{+} cultivars under the LP condition**

```
        Pearson's product-moment correlation

data:  Experiment_3_2_$`S_LP+` and Experiment_3_2_$`PUE_LP+`
t = -0.89502, df = 18, p-value = 0.3826
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 -0.5946214  0.2598402
sample estimates:
       cor
-0.2064152
```

- **Shoot of the *Pup1*-K46^{-} cultivars under the HP condition**

```
        Pearson's product-moment correlation

data:  Experiment_3_2_$`S_HP-` and Experiment_3_2_$`PUE_HP-`
t = 2.4175, df = 18, p-value = 0.02646
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 0.06730102 0.76910177
sample estimates:
      cor
0.4950774
```

- **Shoot of the *Pup1*-K46^{-} cultivars under the LP condition**

```
        Pearson's product-moment correlation

data:  Experiment_3_2_$`S_LP-` and Experiment_3_2_$`PUE_LP-`
t = -0.67234, df = 18, p-value = 0.5099
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 -0.5602365  0.3072849
sample estimates:
       cor
-0.1565194
```

- **Root of the *Pup1*-K46^{+} cultivars under the HP condition**

```
        Pearson's product-moment correlation
data:  Experiment_3_2_$`R_HP+` and Experiment_3_2_$`PUE_HP+`
t = -0.082431, df = 18, p-value = 0.9352
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 -0.4580091  0.4267639
sample estimates:
        cor
-0.01942544
```

- **Root of the *Pup1*-K46^{+} cultivars under the LP condition**

```
        Pearson's product-moment correlation

data:  Experiment_3_2_$`R_LP+` and Experiment_3_2_$`PUE_LP+`
t = -0.28492, df = 18, p-value = 0.779
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 -0.4948530  0.3869903
sample estimates:
        cor
-0.06700517
```

- **Root of the *Pup1*-K46^{-} cultivars under the HP condition**

```
        Pearson's product-moment correlation

data:  Experiment_3_2_$`R_HP-` and Experiment_3_2_$`PUE_HP-`
t = -2.8426, df = 18, p-value = 0.0108
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 -0.8016736 -0.1513839
sample estimates:
       cor
-0.5566166
```

- **Root of the *Pup1*-K46^{-} cultivars under the LP condition**

```
        Pearson's product-moment correlation

data:  Experiment_3_2_$`R_LP-` and Experiment_3_2_$`PUE_LP-`
t = 0.80167, df = 18, p-value = 0.4332
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 -0.279843  0.580496
sample estimates:
      cor
0.1856705
```

Pigment content measurement under full-strength solution

- **Total chlorophyll content**

```
             Df Sum Sq Mean Sq F value Pr(>F)
Treatment     3  1.849  0.6163   3.282 0.0253 *
Residuals    76 14.271  0.1878
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
$`statistics`
    MSerror Df     Mean      CV  t.value       LSD
  0.1877741 76 2.867185 15.1134 1.991673 0.2729203
$parameters
        test p.ajusted    name.t ntr alpha
  Fisher-LSD      none Treatment   4  0.05

$means
                TCh      std  r    LCL     UCL    Min      Max     Q25     Q50     Q75
HP_Pup1-K46- 2.69997 0.42601 20 2.5069 2.89296 1.70915 3.32136 2.51424 2.69507 3.07798
HP_Pup1-K46+ 2.87783 0.37805 20 2.6848 3.07082 2.39422 3.58611 2.59756 2.80328 3.15700
LP_Pup1-K46- 2.78404 0.47907 20 2.5910 2.97702 1.89673 3.53455 2.45524 2.70469 3.17250
LP_Pup1-K46+ 3.10688 0.44403 20 2.9139 3.299868 2.39249 3.9523 2.83331 2.974340 3.4058

$comparison
NULL

$groups
                TCh     groups
LP_Pup1-K46+  3.106884      a
HP_Pup1-K46+  2.877836      ab
LP_Pup1-K46-  2.784041       b
HP_Pup1-K46-  2.699977       b
```

- **Chlorophyll a content**

```
            Df Sum Sq Mean Sq F value Pr(>F)
Treatment    3  1.073  0.3575    3.83  0.013 *
Residuals   76  7.094  0.0933
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
$`statistics`
     MSerror Df     Mean       CV  t.value       LSD
  0.09334343 76 2.437091 12.53632 1.991673 0.1924243

$parameters
        test p.ajusted    name.t ntr alpha
  Fisher-LSD      none Treatment   4  0.05

$means
             Chl a    std  r     LCL      UCL    Min    Max      Q25     Q50     Q75
HP_Pup1-K46- 2.31284 0.29413 20 2.17678 2.44891 1.5533 2.71700 2.19553 2.30120 2.53695
HP_Pup1-K46+ 2.42106 0.26541 20 2.28499 2.55712 2.0605 2.87837 2.20986 2.37443 2.64605
LP_Pup1-K46- 2.38870 0.33396 20 2.25263 2.52476 1.7139 2.93264 2.18304 2.38502 2.66503
LP_Pup1-K46+ 2.62575 0.32385 20 2.48968 2.76181 2.0595 3.20845 2.41146 2.54407 2.80555

$comparison
NULL

$groups
                   Chl a   groups
LP_Pup1-K46+    2.625751      a
HP_Pup1-K46+    2.421061      b
LP_Pup1-K46-    2.388704      b
HP_Pup1-K46-    2.312846      b
```

- **Chlorophyll b content**

```
             Df Sum Sq Mean Sq F value Pr(>F)
Treatment     3  0.312 0.10392    2.31  0.083 .
Residuals    76  3.418 0.04498
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
$`statistics`
     MSerror Df      Mean       CV  t.value       LSD
  0.04497684 76 0.8119426 26.11976 1.991673 0.1335711

$parameters
        test p.ajusted    name.t ntr alpha
  Fisher-LSD      none Treatment   4  0.05

$means
                Chlb       std  r     LCL      UCL      Min     Max      Q25     Q50     Q75
HP_Pup1-K46- 0.743465 0.21272 20 0.649016 0.837914 0.36515 1.06596 0.603181 0.734695 0.98414
HP_Pup1-K46+ 0.844595 0.18967 20 0.750146 0.939044 0.60122 1.25502 0.726057 0.788629 0.92744
LP_Pup1-K46- 0.762066 0.23474 20 0.667617 0.856515 0.41686 1.14415 0.611889 0.704421 1.00658
LP_Pup1-K46+ 0.897642 0.20874 20 0.803193 0.992091 0.59305 1.29762 0.760573 0.813213 1.03491

$comparison
NULL

$groups
                 Chlb     groups
LP_Pup1-K46+ 0.8976427      a
HP_Pup1-K46+ 0.8445959     ab
LP_Pup1-K46- 0.7620667      b
HP_Pup1-K46- 0.7434651      b
```

- **Carotenoid content**

```
             Df Sum Sq Mean Sq F value Pr(>F)
Treatment     3 0.1373 0.04577   3.822 0.0132 *
Residuals    76 0.9101 0.01197
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
$`statistics`
     MSerror Df     Mean       CV  t.value        LSD
  0.01197489 76 1.027275 10.65244 1.991673 0.06892133

$parameters
        test p.ajusted    name.t ntr alpha
  Fisher-LSD      none Treatment   4  0.05

$means
                Car     std   r     LCL     UCL      Min    Max     Q25       Q50      Q75
HP_Pup1-K46- 0.983792 0.105349 20 0.935057 1.03252 0.68712 1.13907 0.947035 0.995487 1.03510
HP_Pup1-K46+ 1.005889 0.096217 20 0.957154 1.05462 0.86314 1.15975 0.922185 0.998740 1.08568
LP_Pup1-K46- 1.024973 0.113649 20 0.976238 1.07370 0.75910 1.18327 0.964198 1.041482 1.08772
LP_Pup1-K46+ 1.094443 0.120942 20 1.045709 1.14317 0.85431 1.33940 1.025622 1.073919 1.12900

$comparison
NULL

$groups
                   Ca     groups
LP_Pup1-K46+  1.0944438      a
LP_Pup1-K46-  1.0249736      b
HP_Pup1-K46+  1.0058893      b
HP_Pup1-K46-  0.9837923      b
```

The correlation between the Pi and pigment content of *Pup1*-K46+ cultivars

- **Pi and Chl a content under the HP condition**

```
        Pearson's product-moment correlation

data:  PIGMENT_270319_1_1_$`Pi_HP+` and PIGMENT_270319_1_1_$`Chl a_H
P+`
t = 1.8408, df = 18, p-value = 0.08219
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 -0.05399417  0.71467523
sample estimates:
      cor
0.3980371
```

- **Pi and Chl b content under the HP condition**

```
        Pearson's product-moment correlation

data:  PIGMENT_270319_1_1_$`Pi_HP+` and PIGMENT_270319_1_1_$`Chl b_H
P+`
t = 0.62018, df = 18, p-value = 0.5429
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 -0.3182497  0.5518405
sample estimates:
      cor
0.1446412
```

- **Pi and carotenoid content under the HP condition**

```
        Pearson's product-moment correlation

data:  PIGMENT_270319_1_1_$`Pi_HP+` and PIGMENT_270319_1_1_$`Caroten
oid_HP+t = 2.1329, df = 18, p-value = 0.04696
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 0.008281076 0.743832022
sample estimates:
      cor
0.4491559
```

- **Pi and Chl a content under the LP condition**

```
        Pearson's product-moment correlation

data:  PIGMENT_270319_1_1_$`Pi_LP+` and PIGMENT_270319_1_1_$`Chl a_L
P+`
t = 0.70304, df = 18, p-value = 0.491
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 -0.3008024  0.5651178
sample estimates:
      cor
0.1634793
```

- **Pi and Chl b content under the LP condition**

```
        Pearson's product-moment correlation
data:  PIGMENT_270319_1_1_$`Pi_LP+` and PIGMENT_270319_1_1_$`Chl b_L
P+`
t = 0.32348, df = 18, p-value = 0.7501
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 -0.3792563  0.5016671
sample estimates:
       cor
0.07602338
```

- **Pi and carotenoid content under the LP condition**

```
        Pearson's product-moment correlation

data:  PIGMENT_270319_1_1_$`Pi_LP+` and PIGMENT_270319_1_1_$`Caroten
oid_LP+`t = 0.50132, df = 18, p-value = 0.6222
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 -0.3429847  0.5322297
sample estimates:
      cor
0.1173468
```

The correlation between Pi and pigment content of *Pup1*-K46^{-} cultivars

- **Pi and Chl a content under the HP condition**

```
        Pearson's product-moment correlation

data:  PIGMENT_270319_1_1_$`Pi_HP-` and PIGMENT_270319_1_1_$`Chl a_H
P-`
t = 0.59136, df = 18, p-value = 0.5616
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 -0.3242816  0.5471453
sample estimates:
      cor
0.1380495
```

- **Pi and Chl b content under the HP condition**

```
        Pearson's product-moment correlation

data:  PIGMENT_270319_1_1_$`Pi_HP-` and PIGMENT_270319_1_1_$`Chl b_H
P-`
t = -0.18989, df = 18, p-value = 0.8515
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 -0.4777805  0.4058375
sample estimates:
        cor
-0.04471338
```

- **Pi and carotenoid content under the HP condition**

```
        Pearson's product-moment correlation

data:  PIGMENT_270319_1_1_$`Pi_HP-` and PIGMENT_270319_1_1_$`Caroten
oid_HP-`t = 1.296, df = 18, p-value = 0.2113
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 -0.172703  0.650561
sample estimates:
      cor
0.2921448
```

- **Pi and Chl a content under the LP condition**

```
        Pearson's product-moment correlation

data:  PIGMENT_270319_1_1_$`Pi_LP-` and PIGMENT_270319_1_1_$`Chl a_L
P-`
t = 1.5511, df = 18, p-value = 0.1383
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 -0.1169188  0.6822259
sample estimates:
      cor
0.3433674
```

- **Pi and Chlb content under the LP condition**

```
        Pearson's product-moment correlation

data:  PIGMENT_270319_1_1_$`Pi_LP-` and PIGMENT_270319_1_1_$`Chl_LP-
`
t = 1.5855, df = 18, p-value = 0.1303
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 -0.1094120  0.6862698
sample estimates:
      cor
0.3500576
```

- **Pi and Carotenoid content under the LP condition**

```
        Pearson's product-moment correlation

data:  PIGMENT_270319_1_1_$`Pi_LP-` and PIGMENT_270319_1_1_$`Caroten
oid_LP-`t = 1.1484, df = 18, p-value = 0.2658
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 -0.2049351  0.6308601
sample estimates:
      cor
0.2612807
```

VITAE

Name Miss Bophal Sok

Student ID 6010220111

Educational Attainment

Degree	Name of Institution	Year of Graduation
Bachelor of Science in Biology	Royal University of Phnom Penh	2016

Scholarship Awards during Enrolment

Royal Scholarship under Her Royal Highness Princess Maha Chakri Sirindhorn Education Project to the Kingdom of Cambodia

Research Fund 2018 by Faculty of Science, Prince of Songkla University

List of Publication and Proceeding

Sok, B., Duangpan, S., Meesawat, U. and Klinnawee, L. (2019). Application of the *Pup1*-K46 marker to evaluate phosphorus-deficient tolerance in lowland rice cultivars from Southern Thailand. *Srinakharinwirot Science Journal*. 35(2).

Sok, B., Duangpan, S., Meesawat, U. and Klinnawee, L. *Application of the Pup1-K46 marker to evaluate phosphorus-deficient tolerance in lowland rice cultivars from Southern Thailand*. Poster session presented at Botanical Conference of Thailand (13th), June 14-15, 2019. Srinakharinwirot University, Bangkok, Thailand.