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Crop Waterstress Management in the Tropics and Subtropics (380c)

Characterizing Effects and Potential Mechanisms of the Major QTL *'Pup1'* in Rice (*Oryza sativa* L.)

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Master of Science Thesis

Hohenheim, August 2011

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Abstract

Quantitative trait loci (QTLs) and candidate genes associated with tolerance mechanisms to abiotic stresses were identified in rice (*Oryza sativa* L.) in the last years.

Phosphorus deficiency is an important factor limiting rice yields under upland or rainfed lowland conditions. But especially in developing countries farmers are facing financial difficulties with increasing costs of fertilizers.

A major QTL improving P uptake rates on P-deficient soils was identified in backcross populations between the *indica/aus* landrace Kasalath (high P uptake), and the *japonica* cultivar Nipponbare (low P uptake). Nipponbare near isogenic lines (NILs), carrying the *Pup1* (<u>P</u> <u>up</u>take <u>1</u>) donor allele from Kasalath, were shown to have higher tolerance of phosphorus deficiency.

Consequently, the objectives of this study were to characterize the effects of the major QTL *Pup1* in NILs under a range of P and water supplies, and to identify possible mechanisms.

A greenhouse experiment with 3 replications was conducted at the International Rice Research Institute (IRRI) in the Philippines. The NILs [14-4 *(+Pup1*) and 14-6 *(-Pup1*) were grown on two different soils (Siniloan and Pangil) with two water treatments (fully irrigated and drought stressed), and 2 nutrient treatments (+P and -P).

+Pup1 NILs had considerably more tillers and were taller than 14-6 (*-Pup1*) under P deficient conditions although this was not significant in all cases. Both NILs grown in the soil from Pangil were taller under drought than under fully irrigated conditions. Furthermore, NILs containing the *+Pup1* QTL showed less leaf symptoms of Fe-toxicity which occurred after some time in the fully irrigated -P treatment in the Siniloan soils.

To additionally characterize *Pup1* we conducted a second pot experiment with *Pup1* NILs and the varieties IR64 and Vandana with the objective to determine the ability of the roots to penetrate hardpans (wax layers).

The NILs did not differ significantly from each other in the results of the root analysis, but IR64 and Vandana performed better than both NILs.

It can be summarized that the *Pup1* QTL did only in some cases improve plant performance. The selected water, fertilizer, and soil treatments caused some unexpected interaction effects modifying the plant response and the effect of the *Pup1* QTL and made it difficult to detect the mechanism.

Keywords: Drought stress, hardpans, NIL, *Oryza sativa*, P deficiency, *Pup1*, QTL

Erklärung

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Hohenheim, 15.08.2011

Signature ____

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Acknowledgements

I appreciate Prof. Dr. Folkard Asch for his supervision of my master thesis.

I heartily thank Dr. Stephan Häfele and Dr. Sigrid Heuer for the possibility to conduct research under their supervision at IRRI.

I also would like to thank Dr. Joel D. Siopongco and Afin Amarante for their help in the greenhouse during the experiments.

Many thanks to “The Boys” who helped to collect the data and kept an eye on the rice plants.

Sincere thanks to Crisanta S. Bueno for the help and brief introduction to root scanning.

Thanks also to Namrata Singh for teaching me DNA extraction and PCR.

Thanks to Jennylyn Trinidad for the help during the lignification studies.

Special thanks to Dr. Tianyi Zhang for the help with the statistical analysis and for the nice accompany to LB square after hard working days at IRRI.

I am greatly thankful to GIZ/Beaf for financial support during my internship at IRRI.

Thanks to Prof. Dr. Andreas Fangmeier for accepting the Co-supervision of the thesis.

Finally, hearty thank to Elena Zondler who supported me during the time while writing the thesis.

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Abbreviations

Ca	Calcium
DAS	Days After Seeding
DM	Dry Matter
Fe	Iron
h	Hours
K	Potassium
LA	Leaf Area
LDM	Leaf Dry Matter
LDW	Leaf Dry Weight
NIL	Near Isogenic Line
ns	Not Significant
P	Phosphorus
Pup1	Phosphate uptake 1
PVC	Polyvinyl Chloride
QTL	Quantitative Trait Locus
QTLs	Quantitative Trait Loci
RDM	Root Dry Matter
RFLP	Restriction Fragment Length Polymorphism
SDM	Stem Dry Matter
SDW	Stem Dry Weight
SLA	Specific Leaf Area
SPAD	Spectral Plant Analysis Diagnostic
ws	Water Stress
ww	Well Watered
+P	Treatment with additional P-fertilizer
-P	Treatment without P-fertilizer
W	Watt
Zn	Zinc

1 Introduction

Rice (*Oryza sativa* L.) is the most important cereal crop for human nutrition in South- and Southeastern Asia. It has been cultivated for thousands of years in the Tropics and Subtropics and is still one of the crops in which scientists try to improve its yield potential and to adapt it to various environments. Although the yields have more then doubled since the 1960's is it necessary to increase it again by 50% to feed the human population until 2050 (Voesenek & Bailey-Serres, 2009).

Phosphorus (P) deficiency is one of the most important abiotic stresses limiting rice yields under upland and rainfed lowland conditions. P is one of the macronutrients for plants and plays a key role in processes such as photosynthesis, respiration, biosynthesis, construction of biomembranes, signal transduction and in genetic information transmission and expression (Fang et al., 2009). A fertilizer application is often too expensive for subsistence farmers or chemical fertilizers are just not available in isolated areas.

Inorganic P is often absorbed by positively charged minerals like Fe and Al oxides and only plant available in low concentrations in the soil solution (Hinsinger, 2001). Even if P fertilizer is applied most of it will be bound in a short time and cannot used by the field crop (Dobermann & Faihurst, 2000). Therefore plants have developed numerous morphological, physiological, biochemical, and molecular adaptations to acquire phosphate from the soil (Raghothama, 1999).

A solution could be in varieties which are adapted to low P-contents in the soil. Some landraces such as the Indian variety Kasalath seem to have developed mechanism allowing higher P-uptake from the soil. To introduce this trait into the modern varieties with high yield potential would be a cost-efficient alternative to P-fertilizers.

2 Literature Review

2.1 Rice

Rice (*Oryza sativa* L.) is the most important crop in South- and Southeastern Asia (Dobermann & Fairhurst, 2000). Together with wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.) it provides more than half of the calories consumed by humans worldwide (Maclean et al., 2002). It belongs to the class of monocotyledons and the family of Poaceae. The two most important species of the genus *Oryza* are *O. sativa* and *O. glaberrina* while the second species is more important in Africa.

The two most important subspecies of *Oryza sativa* are *japonica* and *indica.* The j*aponica* varieties are originally from the Subtropics and have short round grains and are smaller than the *indica* subspecies and are not sensitive to photoperiod. Rice plants from the *indica* subspecies are photoperiod sensitive, have long grains and develop many tillers (Rehm & Espig, 1976). A third subspecies is *javanica* which plays only a local role in some regions of Asia.

Rice is the only cereal that can grow in flooded conditions due to the aerenchyma which supplies the root system with oxygen. The cropping system can be distinguished in four main categories:

- Irrigated rice or paddy rice
- Rainfed lowland
- Upland
- Flood-prone

The irrigated rice cropping system is characterized by continuous flooding of the fields and “drought is generally avoided” (Lafitte et al., 2004). It is the most intensive system with annual grain yields up to 15 t ha^{-1} although average yields vary worldwide between 3 to 9 t ha^{-1}. High inputs of fertilizer and pesticides are often responsible for the high yields.

Rainfed lowland rice is cultivated in bunded fields that are not flooded for the whole part of the cropping season (Maclean et al., 2002). Yields of an annual single crop are only between 1 to 3 t ha^{-1} (Dobermann & Fairhurst, 2000).

Upland rice is often found in valley bottoms and can as well as rainfed lowland be described as a low input cropping system. Both upland and rainfed lowland rice are systems that have major problems related to the amount of water provided by precipitation. In seasons or years with a shortage of water rice farmers face the problem of yield limiting abiotic stress factors like drought and as a consequence also nutrient deficiency (Courtois et al., 2009). Since fertilizer costs for subsistence farmers of low input systems are too high, a possibility to extend yields would be the use of rice varieties with a higher nutrient and water efficiency.

2.2 The role of P in plant nutrition

Phosphorus is one of the essential macronutrients that are necessary for plant growth and development (Ai et al., 2009). It is part of the nucleotide adenosine triphosphate (ATP) which is the molecule in cells providing energy for various reactions. It is also part of nucleic acids, phospholipids and various enzymes.

Phosphorus is mostly available for plants as $H_2PO_4^-$ or HPO_4^{2-}. There is often a high content of P in the soil but the problem of P deficiency is more related to its low availability for plants. In low pH-soils P gets easily bound in Fe- and Al-hydroxides and can get available for plants via resorption (Hinsinger, 2001). Soils with higher pH has the tendency to bind P in Ca-phosphates. Another source of P in the soil is in the form of organic molecules like Phytin (Marschner, 1995).

Due its crucial importance and its disposition to get easily bound to soil particles have plants developed numerous adaption strategies for the uptake of phoshates from the soil (Ramaekers et al., 2010). One of the strategies is the excretion of chelating agents (citric acids) to prompt the solubiliziation of phosphates to the soil solution which is known from *Lupinus albus*. Another is the root induced acidification through an increase of H^+ excretion to the rhizosphere which increases the P-solubilization of calcium phosphates and makes P available.

2.3 Quantitative trait loci

Quantitative trait loci (QTLs) are parts of chromosomes which are related to a quantitative measurable trait.

Quantitative traits in crops such as yield components, flowering time or plant height are of economically importance (Bernardo 2002, Price & Courtois 1999). Molecular biologists, breeders and genetics have been working for years on the detection of QTLs and candidate genes for responses to abiotic stresses like:

- Drought (Bernier et al., 2009)
- N-deficiency (Wang et al., 2009)
- Al-tolerance (Ma et al., 2002)
- P deprivation (Wissuwa et al., 1998)
- Salinity (Ismail et al., 2007)
- Low temperatures (Miura et al., 2011)
- Tolerance to flooding (Neeraja et al., 2007)

The assistance of molecular markers allowed the isolation of regulating genes for tolerance and resistance (Price & Courtois, 1999). The detection of a certain QTL that is responsible for a tolerant mechanism is only the first step. The environment can have a big impact and cause interactions with the QTL (Christou & Klee, 2004).

One of the successful examples for the use of molecular markers to detect QTLs would be the *Sub1* a QTL. Submergence tolerant rice varieties were created with marker assistant breeding strategies located *Sub1* for the trait of submergence tolerance (Neeraja et al., 2007).

There is also the possibility that farmers already benefit from QTLs that are to date undetected in modern varieties. Breeders unconsciously selected to stress dedicated QTLs since e.g. *Pup1* was mapped in a large number of tested upland varieties whether *Pup1* was wanting in lowland/irrigated genotypes (Chin et al., 2010).

2.3.1 A QTL for tolerance to P-deficiency: *Pup1*

The beginning of the detection of *Pup1* was in 1998 when Wissuwa et al. started the mapping of quantitative trait loci (QTLs) for p deficiency tolerance in rice backcross inbred lines. A cross of the *japonica* subspecies "Nipponbare" (modern variety, low P uptake) and the *indica* type "Kasalath" (landrace, high P uptake) created the F1 which was backcrossed to "Nipponbare" to increase the fertility of the *japonica* X *indica* hybrid. After genotyping at 245 RFLP marker loci one of the QTLs with a major effect was detected. Near isogenic lines (NILs) with the genetic background of the tolerant donor parent Kasalath showed three to four times the P uptake of Nipponbare in another experiment (Wissuwa & Ae, 2001b). A higher P use efficiency of 16.6% was also determined comparing P plant content.

This QTL on chromosome 12 which was clearly associated with tolerance to P-deficient soils named "*Pup1*" (*Phosphorus Uptake 1*, later named *Phosphate Uptake 1*) by Wissuwa et al. (2002). The fine mapping of the *Pup1* locus was done in 2009 by Heuer et al. With gene-based markers a high number of rice varieties and breeding lines were screened to find out that *Pup1* is absent from modern irrigation rice varieties and conserved in 50% in the analyzed traditional and upland varieties (Chin et al., 2010).

Previous experiments at IRRI with contrasting *Pup1* sister lines NIL 14-4 (*+Pup1*) and NIL 14-6 (*-Pup1*) showed significantly higher grain number per plant and higher grain weight per plant for NIL 14-4. The determination of shoot dry weight (DW) and root DW showed also a higher value for the *+Pup1* NIL although it was not significant (Chin et al., 2010).

Although rice lines and varieties containing the *Pup1* locus showed an advantage compared to intolerant varieties on P-deficient soils under controlled atmospheric conditions, the environment and condition where *Pup1* is most suitable have not been found yet. One of the problems is the understanding of the "cause-and-effect relations between root growth, external P efficiency and P uptake" (Wissuwa, 2005). The determination of excretion of citrate acid chelating agents and acid phosphatase of the roots showed no significant differences between Nipponbare and *Pup1*-NIls (Wissuwa, 2005).

Most of the NILs used for previous studies have the photosensitive Nipponbare background and field experiments in the Tropics could not be done so far (Chin et al., 2010). To induce flowering and prevent pre-mature flowering all experiments were conducted in greenhouses with external light prevention. The potential mechanism of the *Pup1* QTL is not yet detected and the effect is not well understood.

3 Hypotheses & Objectives

For this study the contrasting near isogenic lines (NILs) *+Pup1* (14-4) and *-Pup1* (14-6) were used since NILs in previous studies showed higher grain yields for *+Pup1* NILs while the effect of the *Pup1* QTL is unclear and not well described.

Consequently, the objectives of this study were to

- characterize the effects of the major QTL *Pup1* in *Pup1*-NILs under a range of P and water supplies, and
- to identify possible mechanisms.

As reference other already established varieties were included to get a response for the level of the abiotic stress caused by low P level soils and water scarcity. Wax layers were used as “hardpans” to check if *Pup1* would give an advantage in making deeper soil layers accessible for the rice plants.

The following hypotheses are adopted:

- *Pup1* causes differences in root morphology. The root morphology caused by *Pup1* is responsible for a better P uptake from the soil and an advantage for plant growth
- As a consequence of better P uptake *+Pup1* plants are better provided with P on P deficient soils and show less growth depression compared with *-Pup1* plants
- Since *Pup1* NILs showed higher yield formation in previous studies it is likely that differences are already visible in the vegetative stage since plants need to first build a source that provides metabolites for the sinks in the generative stage

4 Materials & Methods

4.1 Response of NILs containing *Pup1* under P-deficiency and drought with a focus on the aboveground biomass

4.1.1 Experimental site

The experiment was conducted in a controlled-environment glasshouse at the International Rice Research Institute (IRRI), Los Baños, Laguna, Philippines (14°30'N, 121°1'E, 23 m altitude) during the dry season from January to April 2009. Light period was extended to 14 h per day with installed 100W light bulbs. This guaranteed a proper development of *Pup1* NILs in consideration of the strongly photoperiodic sensitivity similar to their parent Nipponbare. Night and day temperatures during the experiment were between 23°C and 28°C degrees respectively with a relative humidity of about 60%.

4.1.2 Soil and planting material

Two soils were used for different soil treatments in this experiment (Table 2):

- A highly weathered acid upland soil derived from a field in Siniloan, Laguna with a limited P deficiency,
- and a low pH soil from Pangil, Laguna with high P deficiency

Fertilizers were applied before planting the rice seedlings into the pots (Table 1).

- The +phosphorus (+P) treatment was fertilized with 90-60-60 kg ha^{-1} N, P_2O_5, K_2O respectively plus Zn
- The -phosphorus (-P) treatment pots received the same amounts of N, K_2O and Zn as the +P treatment except the P fertilizer.

Fertilizer sources were Ammonium Sulphate (21% N) for N, Solophos (18% P_2O_5) for P, Muriate of Potash (60% K_2O) for K, and $ZnSO_4$ for Zn (35% Zn).

The soil was filled in plastic buckets with a volume of 20 liters.

2 contrasting near isogenic lines (NILs) were used in the experiment. The NIL 14-4 (+*Pup1*) carries the *Pup1* donor allele from the *indica/aus* landrace Kasalath (high P uptake). The NIL 14-6 (-*Pup1*) has the genetic background from the *japonica* cultivar Nipponbare (low P uptake). The two NILs differ in the expression of the OsPupK46-1 kinase, which is expressed in 14-4 whereas 14-6 shows no expression.

Table 1: Computation of fertilizer per plant and pot and applied form of the element

Element	kg per ha	g per plant	g fertilizer per pot with 6 plants	
N	90	0.78	4.69	Urea
P	40	1.33	7.99	Solophos
K	60	0.40	2.40	Muriate of potash
Zn	10	0.27	1.60	Zn sulfate

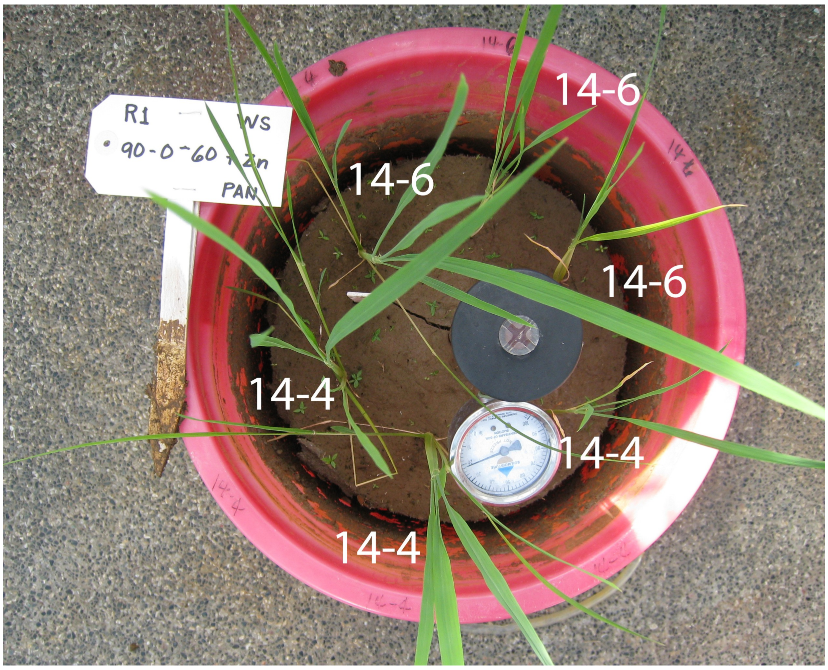

Figure 1: Topview of a pot with 3 plants of each NIL (14-4/+Pup1 and 14-6/-Pup1)

Table 2: Analysis of the soil from Pangil and Siniloan used in the experiments

Soil property	Pangil	Siniloan
pH floodwater	7.10	5.98
EC (dS/m) 1:1	-	0.69
pH soil (1:1)	4.80	3.70
Org C (%)	2.39	2.23
Total N (%)	0.24	0.29
Available Olsen P (ppm)	5.20	18.00
Available Bray 2 P (ppm)	3.20	-
Available K (me/100g)	0.29	0.42
Exchangeable K (me/100g)	0.30	0.34
Exchangeable Mg (me/100g)	4.93	0.20
Exchangeable Na (me/100g)	0.26	0.09
Exchangeable Ca (me/100g)	9.48	0.95
Exchangeable Al (me/100g)	0.17	-
CEC (me/100g)	22.90	16.60
Active Fe (%)	6.04	-
Available Zn (ppm)	4.90	2.20
Soil texture (%)		
sand	2	7
clay	62	67
silt	36	26

4.1.3 Measurements

Seeds of rice were wrapped into wet paper for soaking in Petri dishes for pregermination. After 3 days 2-3 seedlings were sown per hill. Thinning started 10 days after seeding (DAS), whereat the best developed plant per hill was chosen. To compare 14-4 *+Pup1* lines with the 14-6 *-Pup1* lines 3 seedlings of each NIL were grown in the same plastic bucket and had the same soil, water and fertilizer supply.

The spigots at the bottom of the buckets were opened 15 DAS for the drought/water-stress (ws) treatment for simulation of upland conditions. To avoid critical drought stress levels for the ws buckets it was indispensable to supply water from time to time.

The well-watered (ww) buckets were supplied with water regularly. Spigots kept closed to get flooded, anaerobic paddy rice conditions.

The pH of the floodwater was checked for all buckets before planting (Table 2).

From 16 DAS on the plant height was measured with a ruler twice a week as well as the tillers of the plants were counted. Tensiometers (Jet Fill 2725 series) were installed and checked two times to control the water content in the soil (Figure 2)

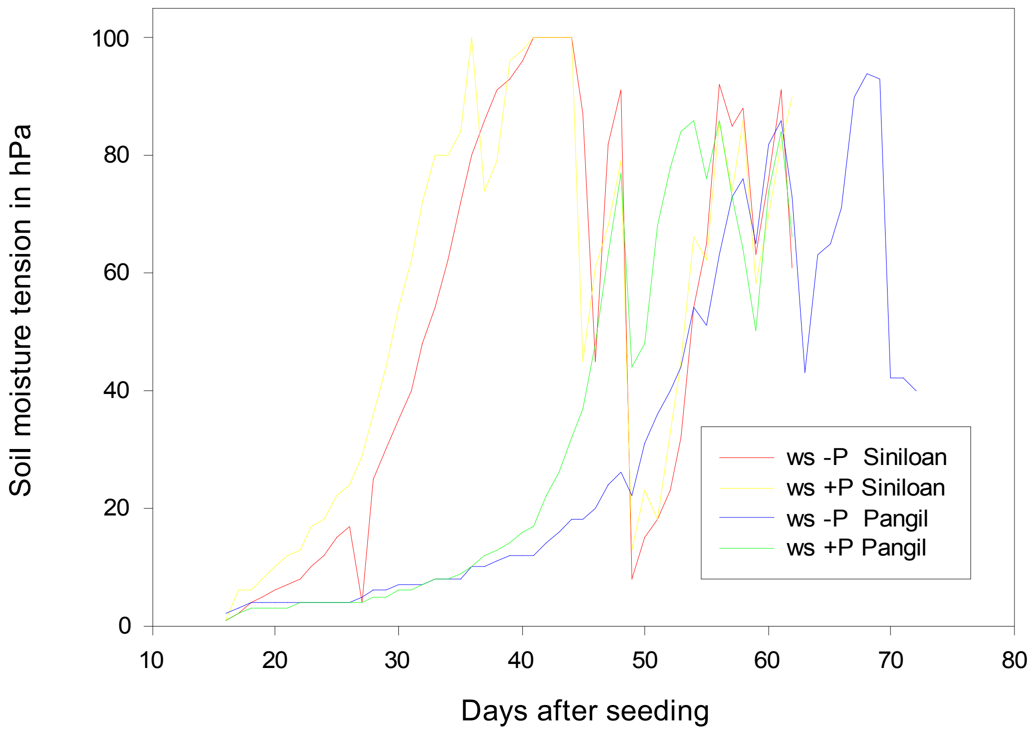

Figure 2: Soil moisture tension over time for the water stress pots (ws) during the growing period for the two soils Siniloan and Pangil with two fertilizer treatments (-P = no P was added; +P = with P-fertilization)

As a proxy for chlorophyll content SPAD values of the second oldest leaf were taken 58 and 61 DAS with Minolta Chlorophyll Meter SPAD-502. The average of 3 measure points per leaf was used for evaluation.

4.1.4 Harvest

The plants were harvested at two different harvest dates (64 DAS and 85 DAS).
P deficiency and drought stress in Pangil soil buckets had a high pressure on plant growth. We decided to harvest the plants of this treatment later as the other plants. The assumption was that differences between the NILs would have been better visible in a later development state for the high stressed plants.
Plants were cut off with a knife above the soil layer. The stems and leaves for each plant were separated and the leaf area (LA) was measured with LI-COR 3100 Area Meter. After that stems and leaves were dried down in a dry oven (NAPCO E series) for 3 days by a temperature of 70°C. The dry matter (DM) of stems and leaves was determined separately.
The ws pots were flooded after the upper parts of the plants were cut off. It was necessary to soak the soil again before it was possible to get out the roots without damaging them.

4.2 Ability of rice roots to penetrate artificial hardpans

4.2.1 Experimental site

The second pot experiment was conducted at IRRI in a greenhouse (BG 14) with controlled environments from 22 March up to 41 (NILs 14-4 and 14-6) and 44 DAS (Vandana and IR64) until harvest.
The illumination to inhibit flowering of the daylight sensitive varieties 14-4 and 14-6 were started 30 DAS from 5 am to 7 am and from 5 pm to 8 pm with installed light bulbs. The pots were placed on a table and after 20 DAS completely randomized. The experimental site included 2 different NILs and to 2 different rice lines, 3 different wax layers with 3 replications of each treatment.

4.2.2 Planting material and soil

The same NILs 14-4 (*+Pup1*) and 14-6 (*-Pup1*) as in the first experiment were used. Additionally we included the rice varieties Vandana and IR64. Seeds were soaked in Petri dishes on wet tissues 3 days before transferring into the soil similar to first experiment.

A cylindrical pot made from polyvinyl chloride (PVC), with 20 cm in internal diameter and 35 cm in height with an inner plastic sleeve, was filled with Pangil soil until 20 cm. The plastic sleeve was used to get the soil out of the pots when plants were harvested. Tensiometers could not be installed because of the wax layers.

Furadan was applied to the soil to prevent problems with nematodes. All pots were fertilized with N, K and Zn according to the first experiment (Table 1). This time no +P fertilizer treatment was included.

As mechanical impedance a 3 mm thick wax layer was then placed flat on the soil, and then topped by 10 cm of soil. Three to four pre-germinated seeds were sown in each pot. 20 DAS the seedlings were thinned to one per pot. Water saturated conditions were maintained in each pot at the beginning. From 20 DAS on no additional water was added until harvest time to simulate rainfed lowland conditions. The pH of the flood water was checked for each pot two days before harvest date (39 DAS for 14-4 and 14-6, 42 DAS for Vandana and IR64).

All pots were flooded 3 days before harvest to soak the soil since it was important not to damage the roots for further analysis and for recording the roots that penetrated the wax layer.

The roots were stored in 75% ETOH by 4°C in a refrigerator until it was possible to scan them (Epson Perfection Scanner V700 Photo). WinRhizo software was used for scanning and working with the files.

4.2.3 Wax layer

To simulate soil compactions a wax-petrolatum system was used in this experiment (Zheng et al., 2002). Wax layer preparation has been described in detail by Siopongco et al. (2009). In summary, wax layers were prepared by melting together paraffin wax and petroleum jelly following the procedure described by Clark et al. (2000). The mixture was poured into molds of 20 cm in diameter and 3 mm in depth before it hardened. The artificial hardpans were designed to provide a contrast in mechanical impedance:

- from almost no impedance (3:97 wax : petroleum jelly)
- to intermediate or middle hard wax (60:40)
- to very hard (80:20) layers

The low impedance hardpan was included as a quantitative check on root penetration below the layer, as described by Clark et al. (2000). The very hard wax layer was used by Botwright Acuña & Wade (2005).

Figure 3: Wax layer in plastic tube before covered with soil

Figure 4: Plastic tube filled with soil and a rice plant of the variety IR64 (37DAS)

4.2.4 Regular measurements

As described in the first greenhouse study the height of the rice plants was measured and the tillers were counted two times a week from 21 DAS on until the time of harvest.

4.2.5 Harvest

Plants were cut with a knife above the root at 39 DAS for 14-4 and 14-6 and at 42 DAS for Vandana and IR64. The leaf area was determined as described in the first experiment. The leaves and stems were dried in a dry oven (NAPCO E series) for 72 hours by a temperature of 70°C. After drying down the plant material the dry matter was determined.

The soil was washed of the roots for each plant separately. The wax layers were observed and the roots that had grown through the wax layers were counted as well as the 'escaped' roots that had grown between the plastic sleeve and the layer.

The roots were scanned after 4 weeks separately for each plant with Epson Perfection V700 Photo Scanner and the files were analyzed with WinRHIZO software. After the scanning process the roots were dried down in a oven (Napco E Series Model 603 Mechanical Convention Oven) at 70°C for 3 days and the root dry matter (RDM) was determined.

4.2.6 Statistics

The experiments were set-up as complete randomized block design although randomization could not be done before 20 DAS.

Data were statistically processed in an ANOVA with SAS and additionally with the R program. When the Equal Variance Test was failed a One Way ANOVA on ranks (Tukey HSD Test at $p < 0.05$) was used instead of Fisher's LSD test in the statistical analysis. This occurred often since the replications showed marked differences.

5 Results

5.1 Comparison of two NILs under drought and P-deficiency in two different problem soils (Experiment 1)

5.1.1 Plant height

Rice plants of the -P- and +P-pots were harvested at two different dates (64 DAS and 85 DAS). A comparison of all plant heights at the first time of harvest is listed in Table 3 and Table 4 for the Pangil and the Siniloan soil treatment respectively. Additionally, the development of the plant height over time is shown in Figure 5 (Pangil) and Figure 6 (Siniloan). The soil treatment had a complete different effect on plant growth and development. Therefore, most results are presented in separate figures or tables for the soils respectively.

The water treatment showed a significant effect under -P-conditions in pots filled with Pangil soil. No LSD-Test could be conducted for the plants grown under +P-conditions due to a large variation in plant heights. P-deficiency combined with flooded conditions led to significant smaller plants compared to P deficiency with drought stress. It seemed that the additional water had a negative effect on plant growth in the high P-deficient Pangil soil. The tallest 14-4 plant with a height of 45.5 cm differed only significantly from the smallest 14-6 plant (40.0 cm) under ww conditions. The other plants of this treatment did not differ significantly from each other. P-deficiency combined with water stress (ws) showed taller plants with measured heights between 56.0 cm (14-4) and 49.0 cm (14-6). An effect of the *Pup1* QTL could not be determined in this case since 14-4 NILs were not taller than the sister line 14-6 (Table 3).

Table 3: Average plant height (cm) 64 DAS for rice plants grown in soil from Pangil. Experiment included the 2 contrasting NILs 14-4 (+Pup1) and 14-6 (-Pup1), 2 nutrient treatments (+P = P-fertilizer was added; -P = No P-fertilizer was added) and 2 water treatments (ww = well watered and ws = water stress). Values show an average of 3 plants. Different letters signify significant differences according to Fisher´s LSD (ns = not significant); * and ** are significantly different.

	Pangil Soil				
		WS	**WW**	**WS**	**WW**
Variety	**Pot no.**	**-P***		**+P****	
14-4 (*+Pup1*)	**1**	**49.5**^{cd}	**43.0**^{ab}	**67.0**	**68.5**
	2	**51.5**^{de}	**44.0**^{ab}	**61.5**	**69.5**
	3	**51.0**^{d}	**45.5**^{bc}	**68.0**	**69.5**
14-6 (*-Pup1*)	**1**	**50.5**^{d}	**42.5**^{ab}	**62.0**	**69.0**
	2	**56.0**^{e}	**40.0**^{a}	**63.0**	**68.5**
	3	**49.0**^{cd}	**44.0**^{ab}	**69.0**	**65.5**
	LSD	**4.87**	**LSD**	**ns**	

Table 4: Average plant height (cm) 64 DAS for rice plants grown in soil from Siniloan. Experiment included the 2 contrasting NILs 14-4 (+Pup1) and 14-6 (-Pup1), 2 nutrient treatments (+P = P-fertilizer was added; -P = No P-fertilizer was added) and 2 water treatments (ww = well watered and ws = water stress). Values show an average of 3 plants grown in the same pot. Different letters signify significant differences according to Fisher´s LSD (ns = not significant). * and ** are significantly different.

	Siniloan Soil				
		WS*	**WW***	**WS***	**WW****
Variety	**Pot no.**	**-P**		**+P**	
14-4 (*+Pup1*)	**1**	**64.0**	**62.5**	**66.0^{b}**	**71.5^{b}**
	2	**62.5**	**63.5**	**61.5^{a}**	**70.5^{b}**
	3	**61.5**	**63.0**	**62.5^{a}**	**70.0^{b}**
14-6 (*-Pup1*)	**1**	**64.0**	**61.0**	**67.5^{b}**	**71.5^{b}**
	2	**61.0**	**62.5**	**62.0^{a}**	**69.0^{b}**
	3	**63.0**	**63.5**	**59.5^{a}**	**66.5^{b}**
	LSD	**ns**	**LSD**	**5.61**	

The soil from Siniloan had only a limited P-deficiency compared with the soil from Pangil. The plant height measured for the rice plants grown in this soil showed no significant difference under -P-deficiency between ww and ws (Table 4). For the +P treatments could significantly taller plants be found if additional water was added (+P/ww). The difference between 14-4 (*+Pup1)* and 14-6 (*-Pup1)* was like in the Pangil soil not significant.

To make the difference in plant growth better visible, the plant height over time for the NILs is shown for Pangil (Figure 5) and Siniloan (Figure 7) separately. To determine a possible effect of the *Pup1* QTL, the values of the figures show an average of all the NILs 14-4 and 14-6 respectively.

For the Pangil soil it could be shown that from on 20 DAS the plants started to differ in their plant height. At the developing stage of 64 DAS all the rice plants of the +P-treatment were harvested with average plant heights between 60 cm and 70 cm.

The drought stress (ws) plants in the -P-treatment showed a faster growth rate between 40 and 78 DAS but at the time of harvest (85 DAS) there were the no significant differences between the plant height of ww and ws anymore.

The 14-4 NILs were marginally taller at the end of the growing period than 14-6 NILS for both the -P- and the +P-treatment.

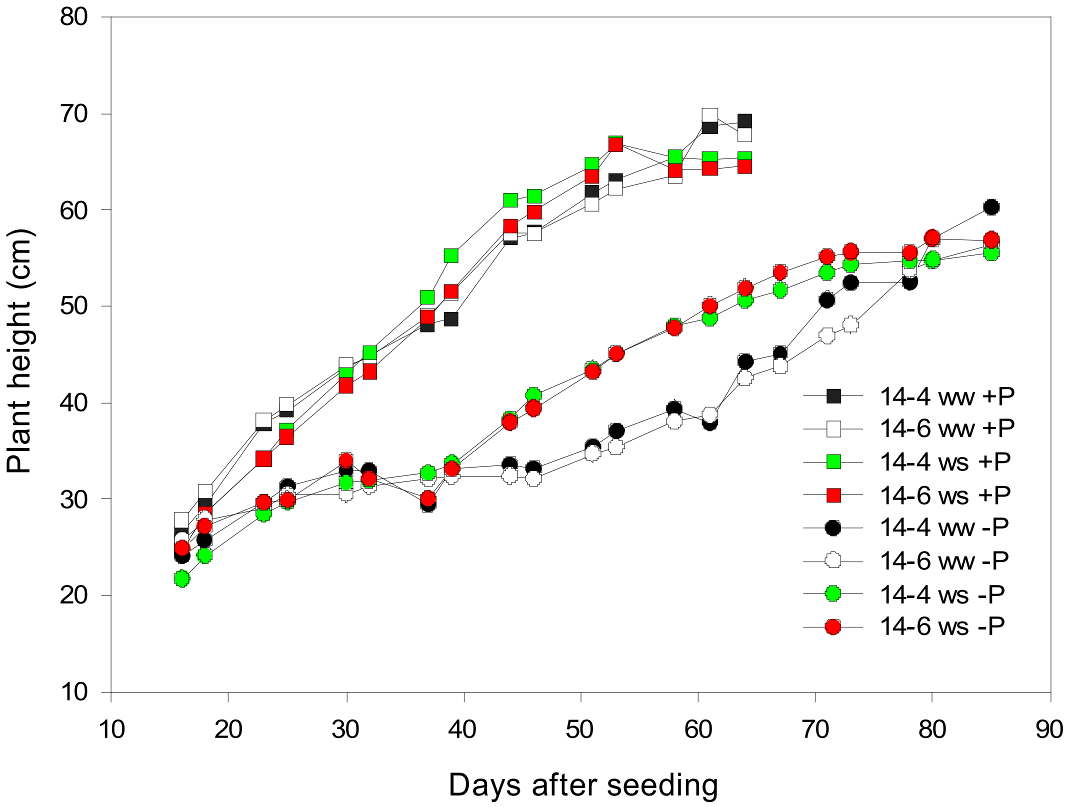

Figure 5: Average plant height (cm) over time for pots filled with soil from Pangil with two P-fertilizer treatments (+P= squares; -P= circles), two water treatments combined with two *Pup1* sister lines 14-4/*+Pup1* and 14-6/*-Pup1* (well watered & 14-4 = black squares or circles; well watered & 14-6 = white squares or circles; water stress & 14-4 = light green squares or circles, water stress & 14-6 = red squares or circles). Data points show an average of 3 pots of the same treatment with 3 plants per pot respectively.

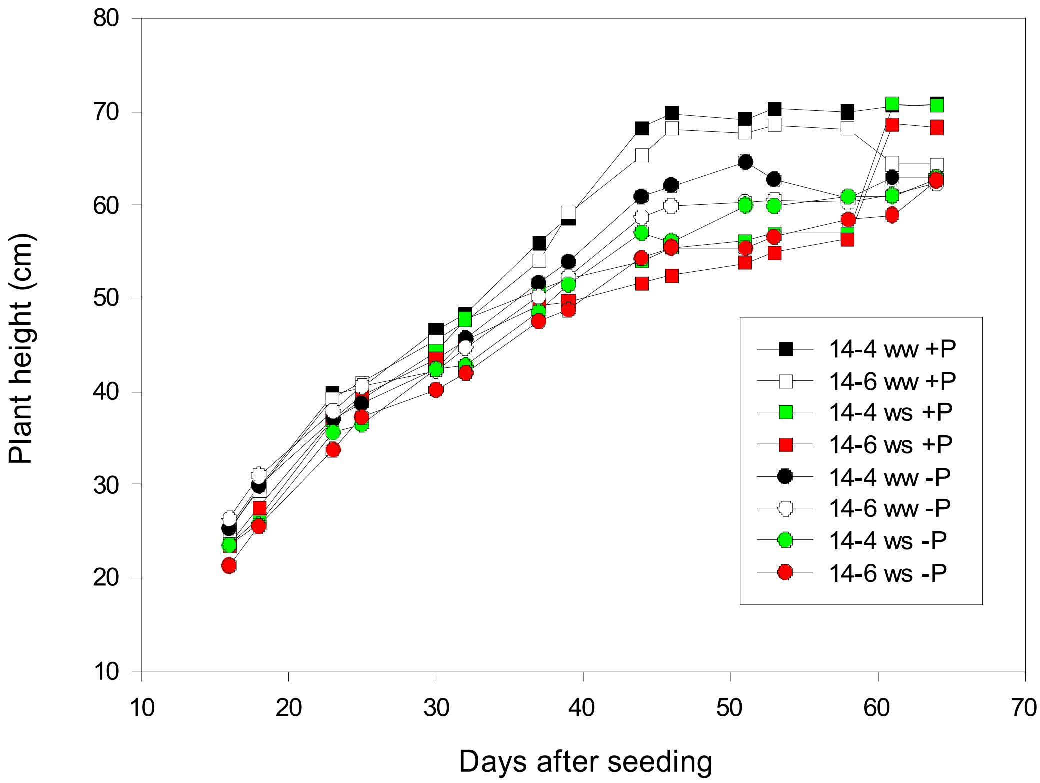

Figure 6: Average plant height (cm) over time for pots filled with soil from Siniloan, Laguna with two P-fertilizer treatments (+P = squares; -P = circles), two water treatments combined with two *Pup1* sister lines 14-4/*+Pup1* and 14-6/*-Pup1* (well watered & 14-4 = black squares or circles; well watered & 14-6 = white squares or circles; water stress & 14-4 = light green squares or circles, water stress & 14-6 = red squares or circles)

Plants grown in soil from Siniloan showed minor differences in plant heights compared with the Pangil soil plants.

Differences in plant height between the treatments were best visible around 45 DAS. Plants with additional water supply (ww) had higher plant heights than drought stress plants independent of P-fertilizer application. The differences in plant height between ws and ww is no longer significant at harvest time in Figure 6. The plants of the drought stress treatment received 2 liters of additional water (Figure 2, page 16) between 50 DAS and 60 DAS since leaf symptoms (rolling) indicate severe drought stress (Price et al., 2002). The supplemental water could be the catalyst for drought stressed plants achieving the same plant height of the ww-plants at the end of the growing period.

5.1.2 Development of tillers

The development of tillers in the Pangil soil depended as well as the plant height primarily on the addition of P fertilizer (Figure 7). The plants of the +P treatment showed a faster tiller development and a higher number of tillers at harvest time than the -P treatment.

Within the +P-fertilizer treatments the water scarcity in the pots seemed to be a stronger constraint for the development of tillers for the 14-6 (*-Pup1*) NIL. The differences between 14-4 (*+Pup1*) irrigated (ww) and drought (ws) showed minor reduction of tillers than the sister line 14-6.

The water treatment had only minor effects on the tiller development when pots drained out over the growing period. The 14-4 NIL in the Pangil soil had a higher number of tillers under -P/ws- and -P/ww-conditions than the 14-6 variety. We could not determine if the differences are significantly different due to high variations between the replications.

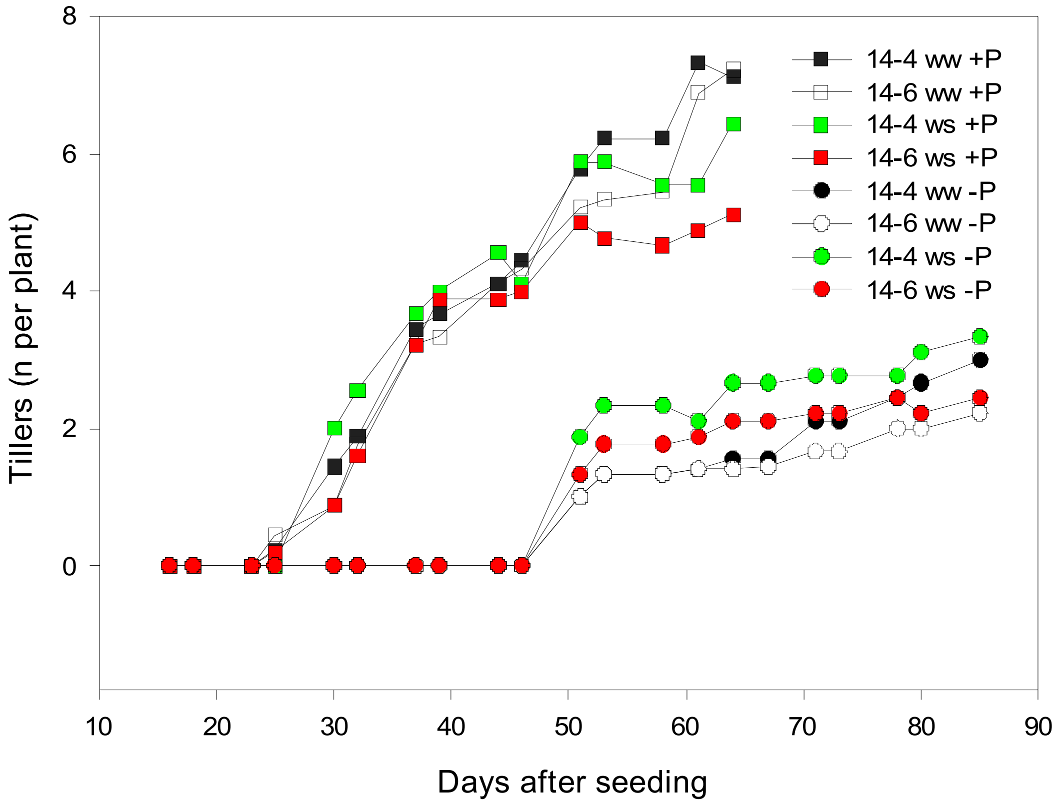

Figure 7: Development of tillers (n per plant) for pots filled with Pangil soil with two P-fertilizer treatments (+P = squares; -P = circles), two water treatments combined with two *Pup1* sister lines 14-4/*+Pup1* and 14-6/*-Pup1* (well watered & 14-4 = black squares or circles; well watered & 14-6 = white squares or circles; water stress & 14-4 = light green squares or circles, water stress & 14-6 = red squares or circles)

In contrast to the plant height differences between the nutrient treatments were observed for the development of tillers. At the end of the experiment both NILs of the irrigated (ww) +P-fertilizer treatment had between 9 and 11 tillers while all plants of other treatments had less than 7 tillers per plant. The drought stress under +P conditions limited the development of tiller much more than under -P conditions.

Fewer tillers were counted when -P-deficiency was combined with drought (ns).

The direct comparison of the 14-4 NILs and the 14-6 NILs showed no significant difference within the same treatments even though the 14-4 line had always a slightly higher number of tillers.

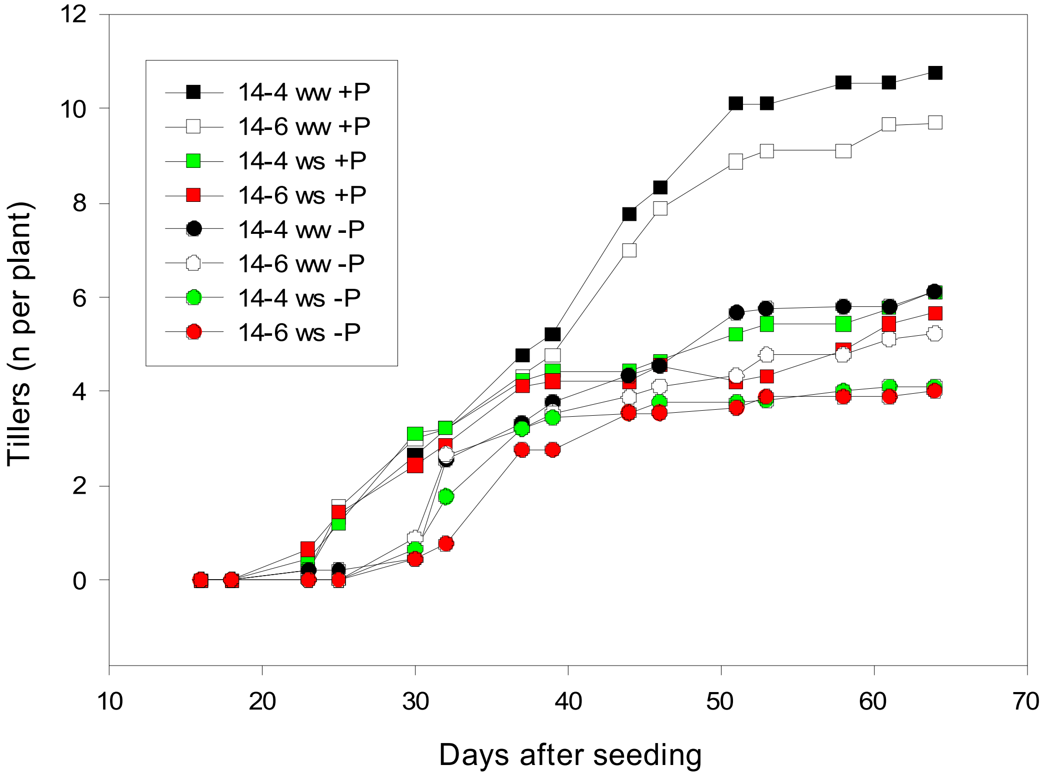

Figure 8: Development of tillers (n per plant) for pots filled with Siniloan soil with two P-fertilizer treatments (+P = squares; -P = circles), two water treatments combined with the two *Pup1* sister lines 14-4/*+Pup1* and 14-6/*-Pup1* (well watered & 14-4 = black squares or circles; well watered & 14-6 = white squares or circles; water stress & 14-4 = light green squares or circles, water stress & 14-6 = red squares or circles).

5.1.3 Dry matter (DM) of aboveground biomass and the determination of the Specific Leaf Area (SLA)

The dry matter (DM) for the plants grown in soil from Pangil is listed in Table 5. The DM under P-deficiency was between 0.9 g and 1.7 g although not significantly different due to high variations among the replications. The P-fertilizer led to a higher DM.

Table 5: Average dry matter (DM) per plant (g) for pots filled with soil from Pangil. Experiment included two nutrient treatments (+P = P-fertilizer was added; -P = No P-fertilizer was added) and two water treatments (well watered / ww and water stress / ws). Values showing an average 3 plants per pot. * and ** are s.d.

	Pangil Soil				
		WS	**WW**	**WS**	**WW**
Variety	**Pot no.**	**-P***		**+P****	
14-4 (*+Pup1*)	**1**	**1.8**	**0.9**	**3.7**	**3.5**
	2	**1.4**	**1.2**	**2.8**	**4.1**
	3	**1.4**	**1.4**	**3.6**	**3.8**
14-6 (*-Pup1*)	**1**	**1.2**	**1.2**	**3.3**	**3.3**
	2	**1.7**	**0.9**	**2.1**	**4.2**
	3	**1.1**	**1.0**	**3.7**	**3.1**
	LSD	**ns**	**ns**	**ns**	**ns**

Plants of the same treatments in the Siniloan soil (Table 6) were less diverse than plants grown in the Pangil soil (Table 5). Therefore, significant differences could be found comparing the DM values (Table 6). The highest DM values ranging from 4.9 g to 5.4 g were found under flooded (ww) conditions when additional P fertilizer (+P) was added. Under P-deficiency additional water had no effect for DM formation and only the two DM values for 14-4 pot no.3 with 3.2 g and 14-6 pot no.2 with 1.8 g showed significant differences.

Table 6: Average dry matter (DM) per plant (g) for pots were filled with soil from Siniloan. Experiment included two nutrient treatments (+P = P-fertilizer was added; -P = No P-fertilizer was added) and two water treatments (well watered / ww and water stress / ws). Values showing an average of 3 plants per pot. Means sharing the same letter are not significantly different.

	Siniloan Soil	**WS**	**WW**	**WS**	**WW**
Variety	**Pot no.**	**-P**		**+P**	
14-4 (*+Pup1*)	**1**	**2.4**^{ab}	**2.7**^{ab}	**3.4**^{b}	**4.9**^{c}
	2	**2.0**^{ab}	**2.6**^{ab}	**2.6**^{ab}	**5.0**^{c}
	3	**2.4**^{ab}	**3.2**^{b}	**2.5**^{ab}	**5.4**^{c}
14-6 (*-Pup1*)	**1**	**2.1**^{ab}	**2.3**^{ab}	**2.4**^{ab}	**4.9**^{c}
	2	**1.8**^{a}	**2.6**^{ab}	**2.1**^{ab}	**5.1**^{c}
	3	**2.3**^{ab}	**2.0**^{ab}	**2.5**^{ab}	**4.4**^{b}
	LSD	**1.3**			

Specific leaf area (SLA) for plants grown in Pangil soil is shown in Table 7 and in Table 8 for plants cultivated in the soil from Siniloan. The high P-deficiency of the Pangil soil combined with drought led in half of the plants to significantly lower SLA (36 $cm^2\ g^{-1}$, 44 $cm^2\ g^{-1}$ and 45 $cm^2\ g^{-1}$) as compared to watered conditions. The additional water had also an effect on the SLA if P-fertilizer was added (+P). The plants benefit from the water saturated soil and could develop a higher leaf area per g biomass.

Table 7: Specific leaf area (SLA) $cm^2\ g^{-1}$ for 14-4 and 14-6 NILs grown in pots filled with soil from Pangil. Experiment included two nutrient treatments (+P = P-fertilizer was added; -P = No P-fertilizer was added) and two water treatments. Values showing an average of 3 replications. Means sharing the same letter are not significantly different. ANOVA was run for –P and +P separately.

		Pangil Soil			
		-P		+P	
Variety	Pot no.	Drought	Well Watered	Drought	Well Watered
14-4 (*+Pup1*)	1	53^{abc}	63^{c}	73^{ab}	110^{e}
	2	36^{a}	75^{c}	69^{a}	90^{cd}
	3	49^{abc}	72^{c}	78^{abcd}	94^{de}
14-6 (*-Pup1*)	1	59^{bc}	56^{bc}	77^{abc}	104^{e}
	2	44^{ab}	67^{c}	86^{bcd}	97^{e}
	3	45^{ab}	69^{c}	72^{a}	93^{d}
	LSD	17		LSD 16	

The Siniloan soil with a medium P-deficiency showed less diverse determined SLA values among the P fertilizer treatments. Since the -P stress was not as high as in the Pangil soil most of the plants did not produce a larger SLA with additional P. SLA did only respond positively if P was added in combination with additional water supply. Under -P conditions for the Siniloan soil treatment the additional water supply did not lead to a higher P supply from the soil and plants did only in 2 cases show significant differences under the -P conditions.

Table 8: Specific leaf area (SLA) $cm^2\ g^{-1}$ for 14-4 and 14-6 NILs grown in pots filled with soil from *Siniloan.* Experiment included two nutrient treatments (+P = P-fertilizer was added; -P = No P-fertilizer was added) and two water treatments. Values showing an average of 3 replications. Means sharing the same letter are not significantly different. ANOVA was run for –P and +P separately.

	Siniloan Soil				
	-P			**+P**	
Pot no.	**Drought**	**Well Watered**		**Drought**	**Well Watered**
1	**79^{ab}**	**82^{ab}**		**114^{d}**	**87^{bc}**
2	**86^{b}**	**85^{ab}**		**80^{b}**	**90^{bc}**
3	**80^{ab}**	**68^{a}**		**79^{b}**	**81^{b}**
1	**81^{ab}**	**82^{ab}**		**62^{a}**	**98^{cd}**
2	**77^{ab}**	**82^{ab}**		**84^{bc}**	**84^{bc}**
3	**84^{ab}**	**73^{ab}**		**83^{bc}**	**88^{bc}**
LSD	**17**		**LSD**	**16**	

5.1.4 Chlorophyll content of leaves

As an indicator of plant health the greenness of leaves were measured with a chlorophyll meter. A SPAD measurement can be used to detect not only deficiency of Nitrogen but also for Phosphate. P deficiency symptoms are dark green leaves (Dobermann & Fairhurst, 2000) and analogous to this high SPAD values can be an indicator for an insufficient P status of the plant.

Significantly different SPAD values could only be found for the -P treatment in the Pangil soil (Table 9) and for the +P/ww treatment for the Siniloan soil (Table 10).

For the plants grown in the high P-deficient Pangil soil plants of the 14-4 (*+Pup1*) NIL showed under -P no differences between drought (35.0 to 39.3) and watered (36.9 to 38.9). The NIL 14-6 (*-Pup1*) showed in 2 of 3 pots plants with significantly darker leaves under -P and drought (41.3 and 41.4) compared with 14-6 plants under full water supply (33.8 to 37.7). There seemed to be a pot effect in pot no.1 under -P/drought/Pangil which shows within the NILs the lowest SPAD values (35.0 for 14-4 and 37-7 for 14-6).

Table 9: SPAD value of the second oldest leaf for plants grown in Pangil soil. Each value shows the average of 3 plants grown in the same pot. SPAD measurement was conducted 58, 59 and 61 DAS. Values in the table show the average of the 3 measurements. ANOVA was run for +P and –P separately. Means sharing the same letter are not significantly different (ns = not significant).

		Pangil Soil			
		-P		**+P**	
Variety	**Pot no.**	**Drought**	**Well Watered**	**Drought**	**Well Watered**
14-4 (*+Pup1*)	**1**	**35.0^{ab}**	**36.9^{bc}**	**40.9**	**39.8**
	2	**39.8^{bcd}**	**36.9^{bc}**	**39.7**	**37.1**
	3	**39.3^{bcd}**	**38.9^{bcd}**	**37.0**	**36.0**
14-6 (*-Pup1*)	**1**	**37.7^{bc}**	**33.8^{a}**	**42.4**	**40.0**
	2	**41.3^{d}**	**35.2^{ab}**	**39.1**	**35.8**
	3	**41.4^{d}**	**37.7^{bc}**	**39.9**	**38.0**
	LSD	**2.9**	**2.9**	**ns**	**ns**

The rice plants grown in soil from Siniloan differed significantly in SPAD values for the P and water treatments respectively (Table 10). Very low SPAD values between 23.5 and 29.4 could be found for ww/-P plants of both NILs. Under P-deficiency and drought higher SPAD values were measured as compared to -P/ww and +P/ww. The NILs did not differ significantly from each other in their SPAD values.

Table 10: SPAD value of the second oldest leaf for plants grown in Siniloan soil. Each value shows the average of 3 plants grown in the same pot. SPAD measurement was conducted 58, 59 and 61 DAS. Values in the table show the average of the 3 measurements. Means sharing the same letter per column are not significantly different. * and ** and*** are significantly different

		Siniloan Soil			
		-P		**+P**	
Variety	**Pot no.**	**Drought***	**Well Watered****	**Drought*****	**Well Watered****
14-4 (*+Pup1*)	**1**	**36.2**	**24.6**	**40.5**	**31.0[b]**
	2	**34.0**	**26.1**	**39.1**	**28.7[ab]**
	3	**35.4**	**28.1**	**39.9**	**26.1[a]**
14-6 (*-Pup1*)	**1**	**35.8**	**23.5**	**40.2**	**32.1[b]**
	2	**37.0**	**24.0**	**39.3**	**28.6[ab]**
	3	**35.8**	**29.4**	**40.9**	**26.6[a]**
	LSD	**ns**	**ns**	**ns**	**3.3**

5.1.5 Effect of Iron (Fe) -toxicity on the NILs

For the plants grown in soil from Siniloan under flooded conditions with additional P-fertilizer leaf symptoms for Fe-toxicity were mentioned from on 57 DAS until the time of harvest (64 DAS). The symptoms were first visible at the second oldest leaves of each plant and affected also the youngest fully developed leaf at about 60 DAS. This was only noticed for the treatment mentioned above and not for any other. Table 11 shows the difference between the varieties 14-4 and 14-6 which clearly showed different damage grades of the leaves (Figure 9 - 12) and differed in the noted number of affected leaves.

The 14-4 plants had less leaves with symptoms (4 out of 17) compared with the *-Pup1* sister line 14-6 with an average of 9 leaves out of 14. The degree of damage was divided into levels of 1%, 5% or 25% of the area of one leaf showing symptoms of yellowish and brown spots. The 14-4 line had a total of 6 leaves with 1% damage, 1 leaf with 5% and 2 leaves with 25%. 7 leaves of the 14-6 plants had damaged at 25%, 2 at 5% and none with a lower level of damage due to the Fe-toxicity.

Since the Fe-toxicity symptoms were only visible in the flooded Siniloan pots with P-fertilizer it is to reason that the soil from Pangil has not a high enough Fe-content to become toxic to the rice plants under flooded condition. The flooded conditions seemed to be the elicitor combined with the additional P-fertilizer as a result of a nutrient imbalance.

Table 11: Number of leaves affected by Fe-toxicity for the NILs 14-4 (*+Pup1*) and 14-6 (*-Pup1*). Values showing the average of 3 plants per pot of each variety with 3 replications. The degree of damage was divided into levels of 1%, 5% or 25% of the area of one leaf showing symptoms. Results base on visual analyzes.

Variety	14-4 (*+Pup1*)	14-6 (*-Pup1*)
Total number of leaves (n per plant)	17	14
Leaves with Fe-tox- symptoms (n per plant)	4	9
Plants with affected leaf area of ~ 1%*	6	0
Plants with affected leaf area of ~ 5%**	1	2
Plants with affected leaf area of ~ 25%***	2	7

Figure 9: Leaf without Fe-toxicity. Treatment: Siniloan soil, water stress, +P-fertilizer

Figure 10: *1% of leaf area affected. Treatment: Siniloan soil, ww, +P-fertilizer

Figure 11: **5% of leaf area is affected. Treatment: Siniloan soil, ww, +P-fertilizer

Figure 12: ***25% of leaf area is affected. Treatment: Siniloan soil, ww, +P-fertilizer

5.2 Results of the wax layer experiment (Experiment 2)

5.2.1 Plant growth and development of tillers for rice plants

The wax layer had neither an influence on the plant height at harvest time nor on the development of tillers (Table 12). However, there were marked differences between the 4 genotypes. The tallest plants were found for the upland variety Vandana with measured heights between 90 cm and 93 cm. There was no significant difference between the NILs 14-4 and 14-6 and the variety IR64. Plant heights were between 63 cm and 65 cm for 14-4 (*+Pup1*) whereas heights for 14-6 (*-Pup1*) were between 61.5 cm and 69.5 cm. IR64 plants reached heights from 61.5 cm to 65 cm.

The comparison of developed tillers for 14-4, 14-6 and Vandana showed values between 5 (14-6/control) and 9 (Vandana/middle hard wax). The variety IR64 had for all wax layer treatments the highest number of tillers with values between 11 and 16.

Table 12: Plant height (cm) and tiller number (n per plant) of the 4 varieties 14-4, 14-6, Vandana and IR64 38 DAS. Rice plants were grown on P-deficient soil from Pangil, Laguna without receiving P-fertilizer under water stress. 3 different wax layers were installed as treatments in the pots: control or soft wax, middle hard wax layers and very hard wax layers. If Equal Variance Test was failed a One Way ANOVA on Ranks (Tukey Test) was used in the statistical analysis. Means in a column sharing the same letter are not significantly different.

	wax layer					
Variety	**control**		**middle hard**		**very hard**	
	plant height	**tillers**	**plant height**	**tillers**	**plant height**	**tillers**
14-4 (*+Pup1*)	**65.0^{a}**	**6^{a}**	**63.0^{a}**	**7^{a}**	**64.5^{a}**	**7^{a}**
14-6 (*-Pup1*)	**61.5^{a}**	**5^{a}**	**63.0^{a}**	**6^{a}**	**69.5^{a}**	**9^{ab}**
Vandana	**93.0^{b}**	**8^{a}**	**95.5^{b}**	**9^{ab}**	**90.0^{b}**	**7^{a}**
IR64	**61.5^{a}**	**16^{b}**	**63.5^{a}**	**13^{b}**	**65.0^{a}**	**11^{b}**
Tuckey test/LSD	**p <0.05**	**6**	**p <0.05**	**4**	**p <0.05**	**2**

5.2.2 Effect of the wax layers on root growth

The average number of roots that penetrated the wax layer (Table 13) were for the soft wax layer (control) between 7 (14-6/*-Pup1*) and 14 (IR64). Lower number of roots were able to penetrate the middle hard wax layer of all 4 genotypes. The lowest number was found for 14-4/*+Pup1* (4) and the highest number for the variety Vandana (9). Although the differences among the genotypes for the soft and the middle hard wax layer seemed to be large, statistical analysis yielded no significantly different results. Only for the very hard wax layer treatment it was possible to determine differences: Less roots were counted that were grown through the hardpan. The range of values were between 5 for Vandana with the highest number of roots while for 14-6 (*-Pup1*) no root penetrated the wax layer.

Vandana and IR64 had a significant higher number of roots penetrating the wax layer than the two NILs 14-4 and 14-6. The difference between 14-4 and 14-6 was not statistically significant for any wax layer treatment.

Table 13: Average number of roots (n per plant) which penetrated the wax layer in the pots of 4 different rice varieties (14-4 /*+Pup1*, 14-4/*-Pup1*, Vandana and IR64). Every value shows the average of 3 plants of the same variety under the same wax layer treatment. Values sharing the same letter per column are not significantly different.

	wax layer		
Variety	**control**	**middle hard**	**very hard**
14-4 (*+Pup1*)	**10[a]**	**4[a]**	**1[a]**
14-6 (*-Pup1*)	**7[a]**	**5[a]**	**0[a]**
Vandana	**13[a]**	**9[a]**	**5[b]**
IR64	**14[a]**	**6[a]**	**2[ab]**
LSD (alpha=0.05)	**ns**	**ns**	**3**

The alternative way for the roots to get access to the soil layers beneath the wax layer was to grow through the space between the pot wall and the layer (Table 14). It was technically impossible to install the layers in the pot excluding this possibility and it seemed that the roots scouted this easier way to grow into the deeper soil layers. The number of escaped roots increased with the hardness of the hardpan.

The NILs did not differ significantly from each other for any of the wax layer treatment.

Table 14: Average number of roots (n per plant) which "escaped" from the wax layer in the pots of 4 different rice varieties (14-4 /*+Pup1*, 14-4/*-Pup1*, Vandana and IR64). Every value shows the average of 3 plants of the same variety under the same wax layer treatment. Values sharing the same letter per column are not significantly different.

	wax layer		
Variety	**control**	**middle hard**	**very hard**
14-4 (*+Pup1*)	**2**^{a}	**6**^{a}	**6**^{a}
14-6 (*-Pup1*)	**2**^{a}	**7**^{a}	**7**^{a}
Vandana	**8**^{b}	**16**^{b}	**21**^{b}
IR64	**6**^{b}	**10**^{a}	**15**^{b}
LSD (alpha=0.05)	**4**	**5**	**6**

5.2.3 Results for the root analysis with WinRHIZO and root dry weight

Table 15 shows the roots surface area (RSA in cm^2 $plant^{-1}$) for all plants after harvest time (38 DAS). The only case where the wax layer treatment seemed to have an effect on the RSA was for IR64 grown as control with a value of 902 cm^2.

The *Pup 1*- NILs differed not significantly in their RSA for any of the wax layer treatments. The varieties Vandana and IR64 had always a significantly larger RSA to the two NILs. Vandana and IR64 differed significantly in their RSA in the control but not when grown in pots with the middle hard or the very hard wax layer.

Table 15: Root surface area (RSA in cm^2 per plant). Experiment included 3 wax layer treatments in the pots of 4 different rice varieties (14-4 /*+Pup1*, 14-4/*-Pup1*, Vandana and IR64). Every value shows the average of 3 plants of the same variety under the same wax layer treatment. Values sharing the same letter are not significantly different.

Variety	wax layer control	middle hard	very hard
14-4 (*+Pup1*)	**244^{a}**	**270^{a}**	**265^{a}**
14-6 (*-Pup1*)	**185^{a}**	**249^{a}**	**192^{a}**
Vandana	**659^{b}**	**668^{b}**	**837^{bc}**
IR64	**902^{c}**	**665^{b}**	**795^{bc}**
LSD (alpha=0.05)	**199**		

Table 16: Average number of root tips (n per plant and pot). Experiment includes 4 different rice varieties (14-4 /*+Pup1*, 14-4/*-Pup1*, Vandana and IR64). Every value shows the average of 3 plants of the same variety under the same wax layer treatment. Values sharing the same letter are not significantly different.

Variety	wax layer control	middle hard	very hard
14-4 (*+Pup1*)	**20701^{ab}**	**22293^{abc}**	**43037^{abcde}**
14-6 (*-Pup1*)	**21862^{abc}**	**27962^{abcd}**	**17140^{a}**
Vandana	**49979de**	**63674^{e}**	**58467^{e}**
IR64	**62337^{e}**	**46586^{cde}**	**56223^{e}**
LSD (alpha=0.05)	**24861**		

The wax layer treatment had no influence on the number of root tips (Table 16). Vandana and IR64 did not differ significantly from each other in the number of root tips except for the very hard WL where more than twice the root tips were counted for 14-4 compared with 14-6. The NILs had both significantly less root tips than Vandana and IR64 (between 49979 and 63674) except for 14-4 (43037) in the the very hard WL treatment where the 14-4 (*+Pup1*) NIL had more than twice root tips compared with the 14-6 (*-Pup1*) NIL.

Table 17: Root dry matter (g plant^{-1}) measured after harvest. Experiment includes 4 different rice varieties (14-4 /*+Pup1*, 14-4/*-Pup1*, Vandana and IR64). Every value shows the average of 3 plants of the same variety under the same wax layer treatment. Values sharing the same letter are not significantly different

	wax layer		
Variety	**control**	**middle hard**	**very hard**
14-4 (*+Pup1*)	**0.19^{a}**	**0.19^{a}**	**0.18^{a}**
14-6 (*-Pup1*)	**0.17^{a}**	**0.16^{a}**	**0.19^{a}**
Vandana	**0.51^{bc}**	**0.44^{b}**	**0.57^{c}**
IR64	**0.67^{c}**	**0.44^{b}**	**0.53^{bc}**
LSD (alpha=0.05)	**0.11**		

Root dry matter (RDM) is presented in Table 17. RDM of the 2 NILs did not differ statistically under any wax layer treatment and was in the range of 0.16 g to 0.19 g. Significant higher RDM than in the NILs was measured for both Vandana and IR64. The significantly highest RDM was measured for IR64 with 0.67 g. Under the middle hard wax layer treatment and the very hard wax layer treatment IR64 and Vandana had no significant difference in RDM. The artificial hardpans did not seem to have an influence on the formation of RDM.

5.2.4 Results for above ground biomass

In addition to the biomass below the surface the aboveground biomass was measured. Above ground biomass values were similar for the 4 genotypes. Vandana and IR64 had more above ground biomass than the two NILs both in leaf dry weight (LDW) and stem dry weight (SDW).

Focusing on the 2 NILs a slightly higher LDW and SDW for 14-4 (*+Pup1*) than for 14-6 (*-Pup1*) was noted in almost every case although the difference is not large enough to be statistically significant.

Differences between the wax layer treatments were significant for Vandana for the LDW and the SDW as well as for the measured LDW for the variety IR64.

Table 18: Leaf dry weight (LDW) and stem dry weight (SDW) in g per plant of the 4 varieties (columns) 14-4 (*+Pup1*), 14-6 (*-Pup1*), Vandana and IR64. Experiment included 3 different wax layer treatments (soft/control, middle hard, very hard) and 3 replications. Values sharing the same letter are not significantly different (ns).

	variety							
	14-4 (*+Pup1*)		14-6 (*-Pup1*)		Vandana		IR64	
wax layer	LDW	SDW	LDW	SDW	LDW	SDW	LDW	SDW
soft / control	0.94^{a}	1.33^{a}	0.86^{a}	1.16^{a}	2.95^{b}	3.47^{bc}	3.24^{bc}	3.49^{bc}
middle hard	1.20^{a}	1.59^{a}	0.87^{a}	1.37^{a}	3.78^{c}	4.38^{b}	2.78^{b}	3.06^{c}
very hard	1.09^{a}	1.38^{a}	1.00^{a}	1.47^{a}	3.19^{bc}	3.49^{bc}	3.16^{c}	3.20^{bc}
LSD	ns	ns	ns	ns	0.77	1.05	0.77	ns

The NIL 14-4 (*+Pup1*) achieved higher LDW than the sister line 14-6 (*-Pup1*) although it was not significant. The LA of the NILs was between 165 cm² and 268 cm². Vandana and IR64 exceeded 14-4 and 14-6 many times significantly over under control, middle hard wax and very hard wax treatments. The only significant different LA among Vandana and IR64 were found for the latter variety grown in pots with a very hard wax layer (1009 cm²). In all other cases the difference between Vandana and IR64 in their metered LA was too small for a statistically difference. The findings suggest that there is no correlation between the wax layer treatments and the development of the LA.

Table 19: Leaf area (LA) in cm² per plant. Experiment includes 4 different rice varieties (14-4 / *+Pup1*, 14-4/*-Pup1*, Vandana and IR64). Every value shows the average of 3 plants of the same variety under the same wax layer treatment. Values sharing the same letter are not significantly different

	wax layer		
Variety	**control**	**middle hard**	**very hard**
14-4 (*+Pup1*)	**194**[a]	**268**[a]	**261**[a]
14-6 (*-Pup1*)	**165**[a]	**201**[a]	**213**[a]
Vandana	**654**[b]	**642**[b]	**746**[bc]
IR64	**773**[bc]	**692**[b]	**1009**[c]
LSD (alpha=0.05)	**294**		

6 Discussion

6.1 Plant height, tiller development and dry matter of NILs (Experiment 1)

The NIL 14-4 containing the *Pup1* QTL showed only in a few cases significant differences to the sister line 14-6. The 3 plants of each replication showed large variations in growth and development. That created large standard deviations and made it difficult to identify differences between the NILs induced by the *Pup1* QTL. In studies with the aim to detect QTLs that confer tolerance to abiotic stresses QTL x environment interaction effect were often ignored (Li et al., 2009). For instance in a study of Bernier et al. (2009) the *qtl12.1* which is linked to tolerance to drought stress showed no effect in a Vandana/Way Rarem population under upland conditions. Differences could also not be detected between Nipponbare and NIL-*Pup1* when grown in small pots that did not allow full root development (Wissuwa, 2005). In the first study of this thesis 6 plants of each NIL were grown in one 20 L bucket. It is likely that there was a strong competition between the rice plants that may have affected the growth and development of the rice plants obscuring any effect of the *Pup1* QTL. Seedlings that could first build up a rooting system and acquire nutrients and water from the soil at the beginning of the experiment could possibly develop better whereas smaller seedlings could not catch up during the growing period since nutrients and water were very limited for 6 plants in a growing period of 85 days.

The aim of the 1.Experiment was to test the *Pup1* sister lines 14-4 (*+Pup1*) and 14-6 (*-Pup1*) under different problem soils representative for the rice cropping areas Pangil and Siniloan (Laguna, Philippines) with the major problem of phosphorus deficiency. The -P-stress of the soil from Pangil affected the plant height of both NILs negatively whereas with P-fertilizer applied rice plants were taller and produced more biomass. Additionally to the soil treatments the water treatment were included to test the performance of the near isogenic lines under water stress. Application of phosphate for half of the plants created a control to the P-deficient soils. The focus of this experiment was on the aboveground biomass.

In a previous experiment Wissuwa & Ae (2001a) could show that the NIL-C443 that contains the *Pup1* locus exceeded the line Nipponbare in P uptake from a P-deficient soil by 170% and by 250% in yield. *Pup1* NILs showed higher P-uptake on P-deficient soils (Wissuwa, 2005) while the physiological analysis of the plants showed higher yields for rice lines with the *Pup1* QTL. To get an idea how *Pup1* influences the plant morphology and physiology it was important to analyze the rice plants before flowering in the vegetative period. Because of this we prevented the plants in both experiments from flowering with the extent daylight period. Since we did not measure the light intensity it is also possible that the short day conditions affected the growth of the NILs negatively.

Since the 2 soils differ widely in their nutrient content, texture and the pH (Materials and Methods, Table 2) the biggest effect on the plant growth parameters was due to the choice of soil. The Pangil soil treatment showed how P-deficiency constraints the plant growth when compared with plants that received sufficient P-fertilizer. At the first harvest date 64 days after seeding the +P treatment combined with flooded conditions showed plant heights between 65.5 cm and 69.5 cm. The rice plants growing on the soil from Pangil under -P conditions achieved only heights between 42.5 cm and 56.0 cm. This difference shows clearly how P-deficiency reduced the growth of rice plants.

A higher number of tillers could lead to higher yields and could be one explanation for the grain weights of *Pup1* NILs in previous studies. In our first experiment the number of tillers was significant lower for the -P treatment (Figure 3). Plants under -P stress could not even develop more than 2 or 3 tillers while the additional P-fertilizer allowed the development of tillers to the extent of 7. Another observation for the Pangil soil treatment was that plant height under the low P-level (-P) seemed not to be additive with the water stress, which will be discussed later.

Rice plants grown in the soil from Siniloan were taller and developed more tillers than plants grown in Pangil soil. The Siniloan soil is from a region with only medium P levels of 18 ppm (available Olsen P, Table 2) compared with the very low P-amounts of 5 ppm in the soil from a field in Pangil. It is very likely that the rice plants in the Siniloan soil could take up more P and therefore developed better.

The limiting factor for the Siniloan soil was rather the water treatment than the P-fertilizer per se. This could be observed for tiller development (Figure 5) as well as for DM accumulation (Table 6). The additional P-fertilizer could not be used by the rice plants under drought. Since P is transported by the water flow to the root surface like most water soluble nutrients, a deficiency of water would reduce the delivery of P to the root. The drought stress in the first experiment limited the number of tillers to maximum values of 6 whereas additional water combined with P-fertilizer lead to plants with up to 11 tillers per plant. That additional P is only a benefit to plant growth if there is enough water available. This could also be shown in the DM (Table 6). Only under well watered conditions the effect of the P-fertilizer was visible since ww/+P plants had in most cases twice the DM than under drought combined with P-deficiency. Wissuwa et al., 2002 could show that there is a strong correlation between the development of tillers and the uptake of P from the soil. Since the tiller numbers did not differ among the NILs in our study it is likely that the *Pup1* QTL did not increase P uptake in the first experiment. Since it was not possible to measure the plant P concentration we used indicators such as the number of developed tillers to estimate differences in the P-nutritional status of the NILs.

That the -P stress was less severe in the Siniloan soil than in the Pangil soil is reflected in the SLA values (Table 7 and Table 8). While the -P stress plants in the Pangil soil could not accumulate more than 59 cm^2 g^{-1} the values for the respective treatment in Siniloan soil pots were at least 77 cm^2 g^{-1}. Additional water lead to a higher SLA especially for the Pangil soil treatment whether under -P as well as under +P conditions. The NILs did only differ in a few cases significantly. An effect of the *Pup1* QTL was therefore not visible.

P-deficiency can cause dark green leaves (Dobermann & Fairhurst, 2000) and it should be possible to detect this with a SPAD-meter which is usually used to estimate the chlorophyll content in leaves via red light absorption (Huang et al., 2008). Higher SPAD values suggest a higher chlorophyll density. If it is used to detect P-deficiency, higher SPAD value should be measured since the K+ATPase, which is responsible for cell elongation is also effected by P-deficiency due to energy deficiency in the cells and less ATP regeneration. P-deficiency tended to result in higher SPAD values in almost every case for the water stress plants in both soils (Table 9 and Table 10). Since the NILs did also not differ in their SPAD values for the treatments it is likely that the *Pup1* QTL could here not enhance the P-nutrition and both 14-6 and 14-4 showed P-deficiency symptoms.

The effect which lead to higher grain yields in previous studies was not visible in the pot first pot experiment. It has to be mentioned that nodules caused by nematodes were found for some roots. Therefore the soil for the second experiment was treated with Furadan to prevent problems with root pathogens. Another error source could be that the drought stress and the P deficiency were too severe in the experiment. Since plants needed more water than expected and the soil moisture potential went down to -100 hPa it was necessary to additionally irrigate also in the drought treatment. This did not result in continuous drought stress scenario but rather in an alternate flooding and drying treatment similar to upland rice systems that might not be the representative environment for *Pup1* conferring an advantage to the rice.

Another unexpected effect was the Fe-toxicity in the Siniloan soil pots which could have a secondary effect on plant performance. The Fe-toxicity symptoms were only visible for the flooded Siniloan soil pots but not for the Pangil soil pots. Fe-toxicity is together with Zn deficiency the most common micronutrient disorder in wetland rice and causes leaf “bronzing” and high yield losses (Becker & Asch, 2005). The 2 NILs 14-4 and 14-6 showed different levels of leaf damage. Although it was not intended to link *Pup1* with a tolerance-effect to Fe-toxicity, the 14-4 NIL that carrying *Pup1* had less leaves with symptoms. The level of damage was also lower than for the sister line 14-6 where (Table 10). The plants were harvested a few days after the symptoms were observed. A chemical analyze to measure leaf Fe-concentrations would have given a confirmation but was not possible in the short time. For conclusive results on the link between *Pup1* and iron toxicity tolerance more studies are needed.

The main difference of *Pup1* NIls in previous studies was the higher grain numbers plant^{-1} and grain weight plant^{-1} for genotypes carrying *Pup1* (Heuer et al., 2009, Chin et al., 2010). The approach was that the differences between the NILs should also be measurable in the vegetative state. The differences between the above ground biomass were too small to draw a conclusion. It seems that the *Pup1* QTL is not effective in all environments and that the interaction of drought and -P-stress makes it hard to interpret the results. It is also possible that *Pup1* changes the growth habit less and that it is less visible in the above ground biomass than expected. In fact, we did also harvest the roots for further analysis with WinRHIZO in the first experiment. The problem was that 6 plants were grown in one pot. It was therefore almost impossible to separate the roots without damaging them. The root samples were also too large to get scanned in the small plastic tray of the scanner. Each root system for each plant had to be divided in more than 20 samples for scanning. Wrong labeling and losses of the samples made it impossible to get data for evaluation and RDM determination.

One of the major objectives was to determine under which conditions the *Pup1* locus confers tolerance to P-deficiency. The difference between 14-4 (*Pup1*) and 14-6 (*Pup1*) in the 1.Experiment should have helped to explain and understand how *Pup1* expresses itself in the accumulation of the aboveground biomass. The influences of the water treatment, the P-fertilizer application, and the soil were much stronger and easier to detect than the differences of the NILs. Chin et al. (2010) describes an experiment with the contrasting NILs 14-4 and 14-6 and came to the very similar observation that there is no significant difference for plant height, tiller number as well as for root length.

6.2 The ability to penetrate artificial hardpans of two NILs and the varieties IR64 and Vandana (Experiment 2)

The results for plant height and number of tillers are listed in Table 11 separately for each wax layer treatment. There was no significant difference between the plant height and tiller numbers between the respective hardpans.

IR64 and Vandana are varieties which are widely established in Asia for different environments. Vandana is a fast growing early rice variety (90 days) from India for upland and hydromorphic conditions and tolerant to drought stress (Bernier et al., 2007). Plants of this variety did not develop more tillers than the NILs 14-4 and 14-6 and less than IR64 while the plant height was much greater than all the 3 other genotypes. Benier et al. (2009) found a QTL for plant height in Vandana even under water stress conditions which could explain why Vandana was able to develop such big plants in our experiment. Vandana has in contrast to the NILs no photoperiodic sensitivity and grew well under short day conditions. New studies showed also that it contains the *Pup1* locus already (Heuer 2011, unpublished).

IR64 is a semi-dwarf variety developed by IRRI and one of the most wide spread

indica varieties of South and South-eastern Asia and adapted to many different environments. The plant height was significant lower than that of Vandana but did not differ significantly from the NILs. Concerning the tiller number IR64 showed the typical characteristic of a semi-dwarf variety with many tillers (11 to 16 all other varieties had less than 9).
Because of their different adaption both IR64 and Vandana were ideal as reference plants for comparison with the NILs for the wax layer experiment. Again, as in the 1.Experiment, no significant differences in plant height and tiller number were observed for 14-4 and 14-6. This could be expected, since the same soil from Pangil without P-fertilizer application was used for the wax layer study. However the plants showed less growth reduction than in the first experiment since this time only one plant per pot was grown.

The wax layer differed in the composition with different amounts of wax and petroleum jelly (Chapter 4.2.3). The soft wax was included as control and did not present a barrier for all genotypes. Compared with the other two layers the highest number of roots were counted for the control hardpan (Table 12). The middle hard wax reduced the number of penetrating roots significantly compared to the control whereas it was reduced again for the very hard wax. All genotypes showed the same tendency of less roots penetrating the wax layer the harder it was.
Vandana differed significantly from the others for the very hard wax layer treatment with 5 roots. The NILs did not differ significantly from each other and neither from IR64. It seems that all genotypes had the same problem with the growth through the hardpan.
Since the layer was placed at 10 cm under the soil surface it seems that the plants found another easier way to get access to the deeper soil layer below it. The alternative way was to “escape” by passing in between the bucket wall and the

wax layer (Table 13). The tendency was the harder the wax layer the more roots took this “alternative” way. The same observation was also described in a study of Siopongco et al. (2009) and by Clark et al. (2000). This relationship is best visualized on the example of Vandana with 8 escaped roots for the control, 16 for the middle hard wax layer and 21 when there was a very hard layer in the soil. This was similarly observed for the other three genotypes although not significant for the NILs. In IR64 and Vandana more roots escaped than in the NILs which did not differ from each other.

The hypothesis was that the *Pup1* locus changes root growth and confers a benefit through better penetration of compacted soil layers, allowing access to deeper soil layers and thus to nutrients and water. Deep rooting rice varieties are traditionally grown in upland rice systems and “are more resistant to drought” (Asch et al., 2005). They can root through compacted soil layers and reach water and nutrient sources there (Clark et al., 2000). Since the NILs have the genetic background of the *japonica* cultivar Nipponbare which is a lowland type (Wissuwa & Ae, 2001a) is it possible that NILs had problems with the hardpan as well as with the dry soil and *Pup1* does not influence the growth of roots.

For a tolerance to P-deficiency it is still unclear if increased root growth is the result of higher external P-uptake or if a high P-uptake mechanisms is responsible for a better development of the root system (Wissuwa, 2005). In the same publication the root surface areas of Nipponbare and a *Pup1* NIL showed a higher increase of the RSA for the NIL but it started to diverge from 40 DAS on. The difference was also only visible under P-stress while plants with sufficient P in the soil did not differ in their RSA. Since the rice plants in our study were harvested 38 DAS is it assumable that differences between the NILs 14-4 and 14-6 cannot be shown in this early growth state. One of the problems of phosphorus in the soil is its immobility and its tendency to sorb to soil aggregates. A strategy to get better access to the P in the soil for plants is to increase their root surface area (RSA).

Since the plants in the second experiment were grown in pots separately and the harvest was only after 38 DAS was it easier to analyze the roots in contrast to the first experiment.

The RSA was determined for the 4 varieties under all wax layer treatments (Table 14). Vandana and IR64 exceeded the RSA of the NILs many times over. As a consequence Vandana and IR64 had more "contact" with the soil and had better access to water and nutrients. Plant height and tiller number were higher for Vandana and IR64 compared to 14-4 and 14-6. For the NILs the RSA value for 14-4 (*+Pup1*) was always higher than for 14-6 (*-Pup1*) but not statistically different in any case. There was again a large variation between replications that caused a large standard variation. As for most other results 14-4 showed a small advantage although it cannot explain a potentially higher P-uptake from P-deficient soils.

The expansion of the RSA would only be one possible strategy to improve the contact with the rhizosphere for a better P-supply for plants. Actually the highest P-uptake rates are in the area of the root tip with its root hairs. Here is the hot spot for P-acquisition. Table 15 shows the number of root tips for each plant. A significantly higher number of root tips was found for IR64 and Vandana compared to the NILs. This fact could increase the quantity for P-uptake form the soil and could be used for the explanation of the good performance of Vandana and IR64. However, it was the aim to get an idea what effect *Pup1* has and what mechanism causes the higher P uptake did the NILs only differ in the number of roots in the case of the very hard wax layer. 14-4 *+Pup1* had 43037 root tips which was more then twice the number for 14-6 *-Pup1* (17140). Since for the control and for the middle hard wax layer the number of root tips was not significant it is still unclear if *Pup1* had an effect to build up more root tips in -P soils than genotypes without *Pup1*. Significantly more roots would in most cases increase the complete subsurface biomass and should probably be measurable in the root dry matter. For the NILs no significant differences in RDM values were found. Chin et al. (2010)

mentioned in a study that "root elongation is independent of *Pup1*" although new studies showed that *Pup1* affects early root growth (Heuer 2011, unpublished). But even if there is a correlation between a large, deep root system with *Pup1* would it not appear to "offer much scope for improving drought resistance in rainfed lowland rice where the development of a hard pan may prevent deep root penetration" (Fukai & Cooper, 1995). Our results suggest that the root morphology is not affected by the *Pup1* QTL. Wissuwa (2005) came also to the conclusion that "enhanced root growth is not co-segregated with the *Pup1* locus" when he compared the root length of the NILs 14-4 and 14-6 seedlings. In an earlier study (Wissuwa & Ae, 2001b) root hair density, root hair length, and root fineness were similar for Nipponbare, Kasalth and the 2 NILs C443 and C498. For Vandana and IR64 there is a clear correlation between the root surface area (Table 15), the number of root tips (Table 16) and the root dry weight (Table 17).

Although the focus of the wax layer experiment was on the development of the root system under P-deficiency, plant height, tiller number, LDW, SDW and LA were also determined.

IR 64 and Vandana showed again that the P-deficiency and the drought treatment were a lesser constrain than for 14-4 and 14-6 which is documented with the higher LDW and SDW in Table 17. There was again no difference between 14-4 and 14-6 and also the wax layer did not effect the formation of the above ground biomass for the NILs. Although the values are significant for the wax layers there is no tendency that the hardness of the wax had any influence on the accumulation of the aboveground biomass. An explanation could be that both IR64 and Vandana were able to overcome the wax layer via the alternative ("escape") way. We cannot conclude that the wax layer influenced the plant growth since leaf and stem DW were not related to the hardness of the wax layer. This proves also true for the comparison of the LA in Table 18 where again 14-4 and 14-6 showed no differences.

7 Conclusion & Outlook

To confirm the results of this study some issues need further clarification:

- Studies to find the perfect environment were *Pup1* unfolds its highest potential should included more than 3 replications for better statistical analysis and water stress should not be too high.
- It seems that *Pup1* does not affect the aboveground biomass in the vegetative stage per se and future studies should focus on the grain filling stage to determine how and when the NILs start to differ. There is still no explanation how the plants in previous studies could develop higher grain yields.
- Varieties that contain the *Pup1* QTL without sensitivity to daylight period would allow field studies in the Tropics where the QTL can be tested directly in P-deficient problem fields.
- Problems with nematodes and root pathogens should be prevented with a Furadan application to the soil.
- In pot experiments the bucket volume should not constrain root development.
- For root analyses other growing substrates should be used than heavy soils which cause root damages when washed off.
- To find out if *Pup1* confers the benefit of increased P-uptake the P content in the biomass has to be measured.

If it was just a contingency that the +*Pup1* NIL showed less Fe-toxicity leave symptoms compared with the -*Pup1* sister line should be clarified in further studies where a set-up with hydroponics can be used.

The tight correlation between P- and water-availability has to be understood for further studies concerning the screening of stress-related QTLs like *Pup1*. One of the major conclusions of this study is that “establishing cause-and-effect relations between genetics and tolerance mechanisms is complicated” (Wissuwa, 2005).

To create rice varieties where the *Pup1* QTL will help to increase yields in upland and rainfed lowland low-input systems more studies are needed. It is not yet understood how the QTL enhances the uptake of P under P-deficiency and under which conditions it has the highest potential (Karwat et al., 2010).

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