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Effects of root zone temperature and P nutrition on Photosynthesis in rice under varying VPD

Thesis prepared for the degree Master of Science

Crop Sciences M.Sc.

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IV. Abbreviations

d	day
DAO	days after onset of treatment
DM	dry matter
g_s	stomatal conductance
N	nitrogen
NAR	net assimilation rate
opt	optimal
P	phosphorus
P_{max}	light-saturated leaf photosynthesis
P_n	net photosynthesis rate
PRI	photochemical reflectance index
Pstat	P status of nutrient solution
P_n	net photosynthesis rate
RH	relative humidity
rs	stomatal resistance
RLGR	relative leaf growth rate
RZT	root zone temperature
SLA	specific leaf area
SWP	stem water potential
Temp	temperature
VPD	vapor pressure deficit
WUE	water use efficiency

V. Abstract

Low root zone temperature and low humidity level might affect the plant growth and photosynthesis. With affordable and handheld devices and proven methods the plant growth and photosynthesis are determined. The photochemical reflectance index (PRI) and the chlorophyll index (SPAD) are used as indicator for the photosynthesis in rice leaves under different treatments and are measured on a weekly basis to compare different root zone temperatures, phosphorus concentration in two *Oryza sativa* (L.) varieties (IR64 and Chomrong). Leaf area, plant dry matter and phosphorus concentration of the leaves were determined at the end of each experiment. In the first experiment three different root zone temperatures (19°C, 25°C and 29°C) and three P concentrations of the Yoshida EDTA solution (0.161mol, 0.323mol and 0.484mol) were tested while in the second experiment low (1.09kPa) and high (2.70kPa) vapor pressure deficit treatments are tested instead of different RZT. Low RZT reduced the leave area in IR64 and Chomrong but high P increased the dry matter in Chomrong at low RZT. The different P concentrations in the nutrient solution resulted in corresponding P concentration in the leaves with a single expectation at the low RZT of Chomrong. The tiller numbers as a far indicator of yield increased with increasing RZT but also high P in Chomrong at low RZT lead to an increased number of tillers. No significant effect of the low RZT on the PRI values can be detected but the end of the experiment SPAD values increased at low RZT. In the second experiment with different levels of VPD the differences between IR64 and Chomrong to their adaptation to specific altitudes and climates is seen clearly. Leave area, leave and plant dry matter is significant reduced in both treatments for Chomrong compared to IR64 under average air temperature of ~32°C and root zone temperature of ~30°C while the VPD and P concentration had no effect. Under high VPD (low air humidity) low SLA were present indicating thicker leaves under water stress. Like in the first experiment the P concentration in leaves is determined by the nutrient solution. PRI under high VPD was reduced indicating lower photosynthetic activity due to water stress. The SPAD values increased over the duration of the experiment indicating thicker leaves.

Keywords: root zone temperature, phosphorus, photosynthesis, ric

1.Introduction

In 2050 the world population will exceed 9 billion and the food production must increase by 70 percent to meet the future needs (FAO.org 2009). This enormous requirement can be met according to the Food and Agriculture Organization of the United Nations but to increase the production is not the solution to gain food security. Recent events in East Africa especially at the Horn of Africa where almost 12 million people are in urgent need for food assistance indicate that food security is not guaranteed globally (United Nations CERF 15 March 2017). Although it is important to note that over the last 50 years there is a significant proportion of the people who suffered from malnutrition due to lack of food access and not the global production (Long et al. 2015). In addition to the missing access to food in some parts of the world the impact of climate change will affect agricultural production globally very uneven. With an increase of 1.8°C to 4.0°C by 2100 the southern hemisphere will see a greater proportion of declining yields and extreme weather events like the current drought in East Africa. The agriculture production in the northern hemisphere in contrast will benefit from the rising temperature. Land areas who not used for agriculture will be potentially suitable for cropping and with the rise in temperature also the growing season will be longer (FAO.org 2009). Although cropping might be possible low soil temperature will be present in the beginning of the cropping season and influence the plant growth.

Shimono et al. (2002) reports that water temperature of rice (*Oryza sativa* L.) grown under flooded conditions can alter various growth processes. At the vegetative period the plant responds to lower water temperature of 23°C with a reduction in tillers, leaf appearance and leaf elongation (Takamura et al. 1960). Matsushima et al. (1964) reported that in early growth stages the water temperature affected rice growth independent of the air temperature. Similar results are reported by Shimono et al. (2002) where the largest reduction in dry matter under low root zone temperature occurred in the vegetative period of rice. Makino et al. (1994) showed that low air temperature affects the photosynthetic rate during the vegetative rate compared to higher air temperature. When the effects of air and water (root zone temperature) are compared the effect of air temperature on the photosynthesis is higher than the root zone temperature. Not only air temperature but also the air humidity is affecting the photosynthesis. Under low air humidity at a given temperature the vapor pressure deficit is higher than under high air humidity based on the calculation of the VPD with the saturated vapor pressure which is given for each degree [°C]. When the relative humidity is below 60% photosynthesis is decreased due to increased stomatal resistance (Horie, 1979). Similar results are presented by Hirasawa et al. (1988) where under increasing vapor pressure deficit the photosynthetic rate and diffusive conductance decreased. Due to modern rice production technologies like early-maturing, N-responsive, semi-dwarf cultivars the cropping intensity and rice yield increased in irrigated lowland in Asia. With increased yields and removal of straw the replacement of nutrients especially Nitrogen, Phosphorus, Potassium and Sulphur must be ensured to maintain the yield level from 4 to 6 t ha^{-1}.With greater demand for food as reported above yields from 8 to 10 t ha^{-1}adjusted fertilization is needed (Dobermann et al. 1998). Yoshida (1981) described that the removal of nutrients from the soil is increased proportionally when the yield increased, although the yield and nutrient removal is strongly dependent on the variety, soil and climate. Phosphorus is an essential element and apart of role as a component for many acids and phospholipids it has a key role in reactions that involve ATP (Taiz et al. 2015). Therefore, it is heavily involved in photosynthesis (Yoshida 1981). Low phosphorus supply to plants reduced their total dry matter and leaf area expansion. When these plants are resupplied with P the dry weights increased continuously (Rao and Terry, 1995). At long term exposure of wheat to low root zone temperature the uptake of P is reduced due to the indirect reduction of nutrient demand by the shoot (Engels 1993). According to Yoshida (1981) the photosynthetic rate is positively correlated to nutrients like P. Increased leaf P content increases the photosynthetic rate following a saturating curve. Makino et al. (1983) found that the amount of P has no influence on photosynthesis until there are visible symptoms of P deficiency. Taiz et al. (2015) described stunted growth and dark green coloration as characteristic symptoms of P deficiency. Similar results as Makino et al. (1983)were found by Rao and Terry (1995)that the photosynthetic rate under low P-treatments sugar beets was reduced to smaller extent than the leaf area and total plant dry matter.

Under unfavorable conditions like cool seasons farmers tend to apply more P fertilizer to their crops than in optimal growing condition (Zhou et al. 2009). Although there is no full explanation to this practice, Zhou et al. (2009) found that plants with high P supply under low temperature conditions produced the highest shoot dry weight and P content in leaves. Results from Starck et al. (2000) indicate that plants at low temperature need higher P fertilization to sustain photosynthesis. The practice of increasing P supply under unfavorable conditions and its effect on the plant of increased P is unclear compared to low P plant treatments.

In this thesis, the effect of different P nutrition of rice plants under different root zone temperatures and different vapor pressure deficits on growth and photosynthetic activity is determined. Following hypothesis are used:

1. Lower root temperature inhibits the growth, the uptake of phosphorus and has an effect on the photosynthesis because phosphorus is directly involved in the regulation of the Calvin cycle and triose phosphate export from the chloroplast. With an increasing amount of phosphorus in the nutrient solution the negative effect of low root temperature might be mitigated.

2. Tillering is highly impaired by nitrogen or phosphorus deficiency and the nutrient status of the plant should be related to the number of tillers. In low P-treatments a lower number of tillers should be produced compared to the optimum and high P-treatments.

3. Under high vapor pressure deficit higher content of phosphorus in the nutrient solution and better availability to the plant helps to control the loss of water through transpiration. The low relative humidity under high VPD will influence

the photosynthetic activity, P concentration in the leaves and dry matter through water stress.

2. Literature overview

2.1. Tested varieties in the experiments

In this experiment two varieties IR64 and Chomrong also called Chhomrong were grown and examined. The International Rice Research Institute (2017) stated that *Oryza sativa indica* is the mayor type of rice grown in the tropics and subtropics. Countries where *O. sativa indica* is mostly grown includesPhilippines, India, Pakistan, Java, Sri Lanka, Indonesia, central and southern China, and African countries. The IRRI described the rice sub-species Indica as rice with broad to narrow, light green leaves and with a tall to intermediate plant structure. They also state that Indica plants do tiller profusely. *Oryza sativa (L.) indica* cultivar IR64 is a short duration rice crop originally from the Philippines (Shrestha et al. 2011). Dingkuhn and Miezan (1995) classified IR64 as a Type III which performs good in hot-dry and wet seasons. According to Wu et al. (2005) IR64 is the most widely grown Indica rice cultivar in Southeast Asia and it has many desirable agronomic characteristics. These characteristics include wide adaptability, high yield potential, tolerance to multiple diseases and very importantly its grain widely accepted(Wu et al. 2005). Fitzgerald et al. (2009) reported that the shape, uniformity and translucence of rice grains are decisive aspects of quality for the consumer. With this characteristics IR64 is an ideal genotype for identifying mutational changes in traits of agronomic importance.

The second variety used in this experiment is *Oryza sativa* (L.) japonica Chomrong. Japonica rice is the second major eco-geographical race of *O. sativa (O. sativa japonica)*besides Indica. Japonica rice is a group of rice varieties from northern and eastern China which are grown extensively in cooler zones of the subtropics and in temperate zones (IRRI.org 20/03/2017). It is described by the International Rice Research Institute as narrow, dark green leaves with medium-height tillers and short to intermediate plant stature. *Oryza sativa* L. spp. temperate japonica (type traditional) cultivar Chomrong is a short duration rice crop originally from Nepal (Shrestha et al. 2011). Sthapit et al. (1995) found that Chomrong is adapted to altitudes from 1400 up to 2000m and is cold tolerant at all growth stages. For the rice farmers at these high altitudes (>1000m) of Nepal the susceptibility to cold, blast and low yields are the major problems. At the location Chomrong (2000m) in Nepal the cold air and water caused a high degree of spikelet sterility and a natural high disease pressure from bacterial sheath brown rot disease and blast (Sthapit et al. 1995). Although Nepal has many cold-tolerant and genetically diverse rice landraces this potential is not been used to a high degree in the national breeding programs. Sthapit et al. (1995) reported that Chomrong and Palung-2 are the only two cultivars which are released as chilling-tolerant cultivars suitable for the high hills out of 28 rice cultivars recommended by the National Rice Research Program (NRRP).

Apart from being different from sub-species the ability to withstand cold temperature and chilling stress is the main difference between IR64 and Chomrong. Chomrong is cold tolerant at all growth stages (Sthapit et al. 1995) and IR64 is supposed to be cold sensitive. Dingkuhn and Miezan (1995) listed IR8,IR50 and IR64 as originally from the Philippines and Sthapit et al. (1995) described IR20, IR36 and IR8 as cold sensitive. They are adapted to altitudes ranging from 100m to 400m and even 700 m.

2.2 Influence of P on plant growth and photosynthesis

Phosphorus in form of phosphate (PO_4^{3-}) is an integral component of important compounds in plant cells and nucleotides which are part of the plant energy metabolism like ATP (Taiz et al. 2015). Phosphate is taken up in form of $H_2PO_4^-$ by proton-coupled P transporters (Marschner 2012). The uptake rates of wheat and maize under long term low RZT are not primarily determined by the temperature effect on the uptake process but rather on the shoot demand for P. The plants under low RZT adapted their P uptake to the internal P status and the translocation rate of P showed significant correlation to the shoot demand. (Engels, 1993). When phosphorus is not available to the plant in sufficient amount typical symptoms for phosphorus deficiency like stunted growth of the whole plant and dark green coloration of the leaves occur. In some species, excess anthocyanins are produced under phosphorus deficiency and results in purple coloration of some plant organs like the base of the stem (Taiz et al. 2015).

Significant reduction of leaf area and dry weight of plant organs by low P-treatments is reported by Rao and Terry (1989), Abadia et al. (1987), Rao and Terry (1995) and Starck et al. (2000). Although low-P treatments do reduce the leaf area and dry matter the photosynthesis Abadia et al. (1987) showed in sugar beet that low-P does has little effect on photosynthesis. Similar results were presented by Makino et al. (1984) with rice that the photosynthetic rate of the treatment without P was the same as the control treatment. In C_3 plants like *O. sativa* (L.) the primary step of photosynthetic CO_2-assimilation the RuBP carboxylase function as a catalyzer (Makino et al. 1984) Therefore a highly positive relationship between RuBP carboxylase activity and the rate of photosynthesis was shown by Makino et al. (1983). Makino et al. (1984) reported that phosphorus does not affect the content of RuBP carboxylase content directly while there are reports by Jacob and Lawlor (1993) that long term P-deficiency leads to reduction in RuBP production. Short-term P deficiency had no effect on the photochemical apparatus in contrast to long term P-deficiency which resulted in photoinhibition and depression of the PSII function (Xu et al. 2007).Rao and Terry (1995) found similar results than Abadia et al. (1987) that the major effect of low P is the decrease of leaf area expansion rate and shoot growth rather than the decrease of photosynthesis per area which is only mildly affected. It is important to compare the results of experiments where at least some P is used and plants are not subjected to P-deficiency like in no-P treatments.

2.3. Advantages and disadvantages of a hydroponic system

Compared to soil culture or field trials in a hydroponic system the researcher can easily manage the plant nutrients, control the pH value and the concentration of micro and macro nutrients (Sonneveld and Voogt, 2009). The management of plant nutrients and therefore also the growth of plant is easier in hydroponic or solution culture than in soil because there is no risk of soil borne disease or pests (Heshey 1994). As described by Sonneveld and Voogt (2009) in many experiments which are done in hydroponic systems the solution is considered as soil. With a hydroponic system, it is possible to identify the effect of each nutrient on the quantity and quality of yield because in a hydroponic system there are far less interaction by different soil properties (Torabi et al. 2012). For example Xu et al. (2007) tested the effect of phosphorus deficiency on the photosynthetic characteristics of rice plants in pots with nutrient solution. Naoko and Jun (2010) described the soilless culture as a simplified experimental method compared to the heterogeneity of the materials and organisms in the soils and the interaction between them. Based on the disadvantages found in soil cultures the hydroponic system is developed und used in many experiments. Although the hydroponic system is a great tool for scientific research in plant nutrition the interaction in soil between the elements and organisms must be done in soil experiments (Naoko and Wasaki, 2010). Apart from effects of single nutrients on plant growth hydroponic systems can be used to simulate soil water deficit. When dry soil is used to simulate soil water deficit or drought it may restrict the access of plants to essential elements like P or N and suffer deficiency while in hydroponic system this problem does not occur (Munns et al. 2010). Torabi et al. (2012) reported that the hydroponic system is the most appropriate and profitable method to investigate abiotic stress on the plants. Abiotic stress could be temperature, humidity but also physical and chemical factors like salinity.

Further advantage of a hydroponic system is that the hidden parts of the plants the root growth and development over time under different conditions can be observed in detail. For example, the root anatomy, morphology and shoot to root ratio under specific condition can be studied. (Torabi et al. 2012). Torabi et al. (2012) further reports that also nutrient deficiencies in plants without the interferences of unwanted factors like soil, microorganisms and toxic elements can be studied. Another field of research where hydroponic systems are applied is the screening and selecting of tolerant plants to heavy metals (Zabtudoswka et al. 2009). In hydroponic systems, the concentration of heavy metals can be chosen but there are reports that the plants react differently under hydroponic systems and soil cultivation. Dickinson et al. (2009) stated that the transfer of results from a hydroponic system to field conditions is not possible because of the differences of these two systems.

When the limitations of hydroponic systems are known, than transfer is certainly possible. The hydroponic systems allow researchers to conduct experiment with plants in climate chambers to simulate the effects of climate change like higher CO_2 on the plant growth. Hydroponic systems in combination of climate chambers can imitate temperature conditions and their effects on plants can be tested independent of the season.

3. Material and Methods

3.1. Plant cultivation and varieties

Oryza sativa (L.) spp. indica IR64 and *Oryza sativa*(L.) spp. temperate japonica cultivar Chomrong also referred as Chhomrongare grown in a greenhouse at the University of Hohenheim in a hydroponic system using the Yoshida EDTA nutrient solution. To test the parameters of low root zone temperature, different phosphorus nutrition and air humidity two experiments were done.

The seeds were obtained from seed bank at the Institute of Agricultural Sciences in the Tropics (University of Hohenheim). For the first experiment the seeds were germinated in the dark environment on moist paper and transplanted into 50% Yoshida solution after 12 days. During the stage of 50% Yoshida solution the cooling system was set to 20°C. After 19days, the plants were transplanted into 100% Yoshida solution and the temperature treatment(setting on Teco units: 24°C, 19°C and 14°C) were started at 26 days after germination. Due to problems with the pH-value of the Original Yoshida solution the Yoshida EDTA solution was used after 22 days after germination until the end of the experiment. The first measurement was done 42 days after germination due to the problems named above. Measurements are described with days after onset of treatment (DAO). Before the second measurement the plants were treated with insecticide due to spider mites (*Tetranychidae*). After the successful treatment, the experiment was continued because no damage to the leaves were found.

In the second experiment the plants were transplanted 10 days after germination into 50% Yoshida EDTA solution and into 100% Yoshida EDTA solution 17 days after germination. The plants were placed into the VPD-chambers with high and low VPD and the first measurement was done 38 days after germination.

Figure 1: Germination plates with seed from variety Chomrong (left) and IR64 (right)

2.2. Experiment set up

To test the effects of three different root zone temperature in the first experiment a block design was used. Each plant was placed in a pot containing 1 liter of nutrient solution and one block contained 24 pots. Four replications for every phosphorus level and variety was used. Inside each block the plants and P-treatments were completely randomized. The pots are arranged in a row and connected with tubes to the pots next to it. Inside the pot the tube circled twice to increase the cooling and heating effect. The whole water cooling cycle consists out of two water circles. The smaller water pump (Type RESUN submarine water pump Qmax: 3000l/h)cycled the water from the water tank containing ~ 50L water to the TECO unit (TR series aquarium chiller) which regulated the water temperature inside the water tank. A second bigger pump (Type Variolux Qmax:4000l/h)moved the water through the tubes which were inside the pots and modified the nutrient solution temperature. The pumps and TECO units were working 24h a day to keep the temperature of the nutrient solution at the desired temperature. This indirect cooling and heating system had no direct contact with the nutrient solution (Fig.2) and with covering the pots with aluminum foil the growth of algae was avoided (Fig. 4).

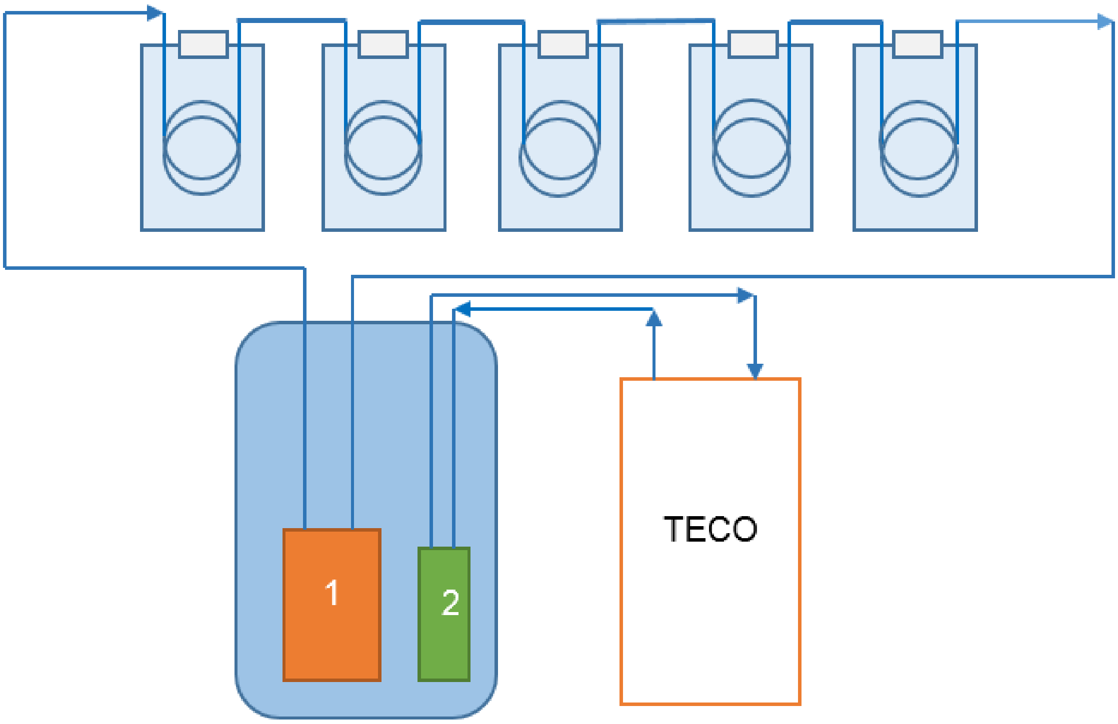

Figure 2: Experimental Setup for each temperature treatmentwith pump (1) for circulation of water and pump (2) for second circle for water temperature regulation. Heating or cooling of water is done by the TECO unit.

Figure 3: Experimental Setup of experiment 1 with three blocks for different root zone temperatures, blue arrows (cool) and red arrow (heated) shows cooling cycle. 1: low temperature; 2: medium temperature and 3: high temperature

Figure 4: Experimental Setup of experiment 1with additional lighting and aluminum cover

The experiment was done in the greenhouse and therefore the changing and high air temperature affected the temperature of the nutrient solution. Due to the high air temperature, the TECO units are limited in their capabilities to maintain the water temperature for the water cooling system. Tinytac type TGP-4500temperature logger with a sensor were placed at the pot in the middle of each block. In addition, temperature measurements were made with Tinytac TV-4020 at the 1,6,12,18 and 24 pot to test if there is a gradient inside the block. Multiple measurements showed that in the there is a gradient of 1,275°C, 0.9°C and 0.475°C at the low, medium and high RZT respectively.

Additional lighting was provided with 400W lamps (Professional Lighting Type SON-K-400)during the 12-hours photoperiod from 8 a.m. to 8 p.pm. above the plants.

In the first experiment the plants are lined up under the lights and every second light were turned on to avoid additional heating up of the nutrient solution. To avoid any impact on the photosynthetic activity every third day the lights with even and uneven numbers were changed to give even conditions for growing conditions. In addition to the change of lamps the exposed tubes were covered with aluminum sheets to reduce the impacts of the heat.

For the second experiment the pots with plants were placed inside two smaller chambers covered with plastic foil to separate these chambers from the greenhouse environment. Each phosphorus treatment had four replications and these replications were placed randomly inside the VPD chambers. The nutrient solution was replaced weekly and the pots were randomized. A small fan in the wall of the chamber was used to circulate the air inside the chamber and avoid any effect of placement. Inside this VPD chambers the relative humidity was automatically set to the desired VPD value. To measure the temperature and relative humidity tinytac logger type TGP-4500 was used in each chamber. As in the first experiment additional lighting was provided with 400W lamps (Professional Lighting Type SON-K-400) during the 12-hours photoperiod from 8 a.m. to 8 pm. from the outside of the chamber. To reduce heat buildup inside the chambers only every second lamp was used to have distance between the lamps of ~45 cm.

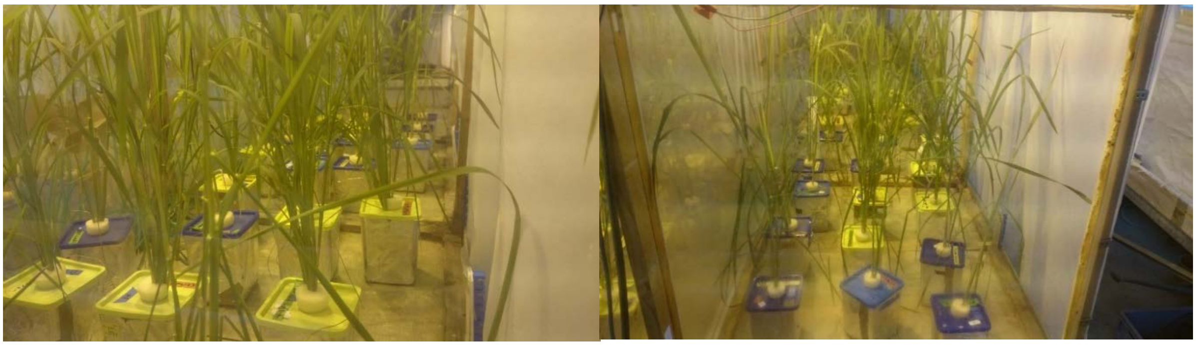

Figure 5: VPD chamber of experiment 2 with Chomrong and IR64 rice plants in pots marked with color corresponding to their phosphor concentration in nutrient solution.

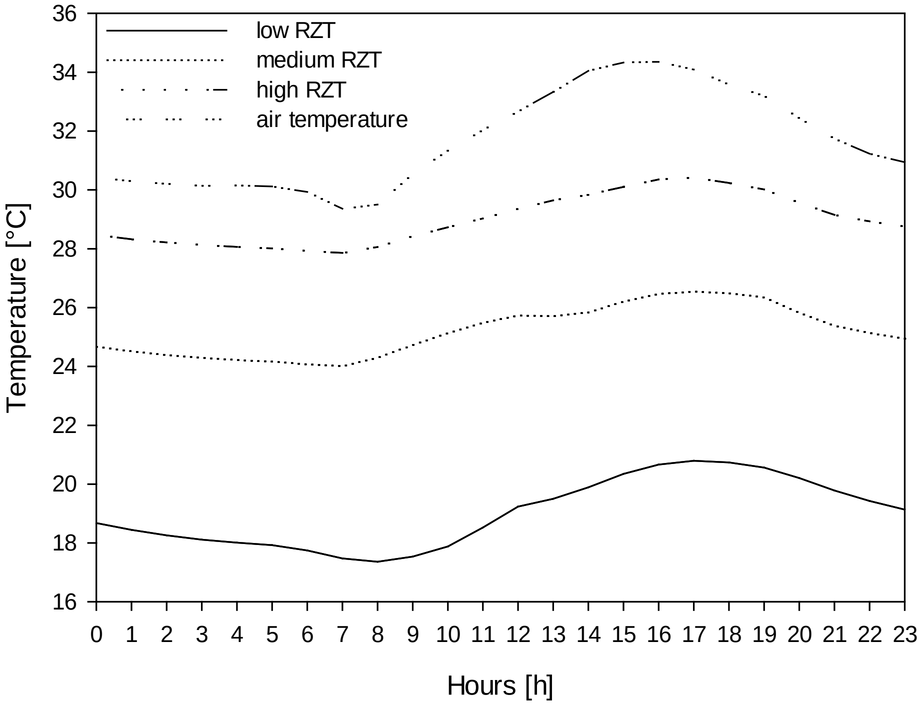

Figure 6: Average temperature per hour of air and different root zone temperatures during a day.

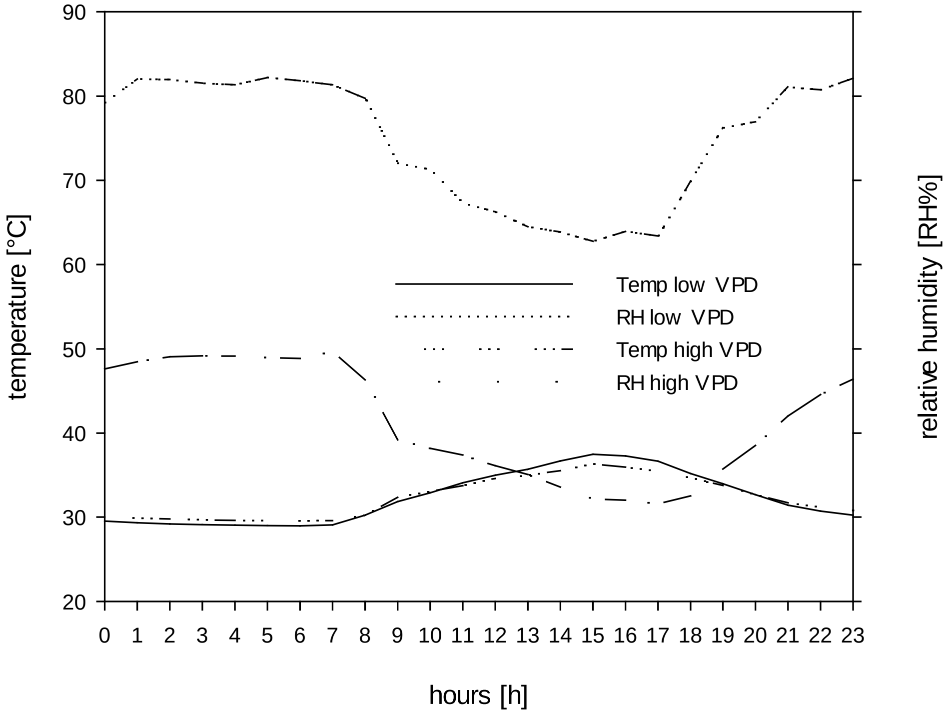

Figure 7: Average hourly air temperature and relative humidity during a typical day in the VPD chamber.

2.3. Nutrient solution

In both experiments the nutrient solution was changed weekly and was created on demand. Seven smaller flasks containing the nutrients listed in table 1 are used to store the stock concentrations. For each P-treatment the nutrient solution was created individually and only the amount of P [ml] was changed. When 30l of nutrient solution was used than 15ml, 30ml and 45ml of P was used for the 50%,100% and 150% P-treatments. The molar mass of P is displayed in table 1. In this experiment the P-treatment with 0.323 mol P is the control treatment and is called optimal. This treatment is called optimal based on the assumption that the amount of P recommended by Yoshida et al. (1976) is the "optimal" P nutrition. The low P-treatment is 0.161mol P and the high P-treatment 0.484mol P.

All other nutrients are used in the same amount in every treatment and experiment. The pH-value was set to 5.5 – 5.8 to have the same conditions for plant roots and uptake. For adjusting the pH value the WTW pH 340i instrument was used. Each nutrient solution was created in a designated container with deionized water for the whole experiment.

Table 1: Yoshida EDTA solution. Modified after Yoshida et al. (1976)

Element	Chemical	Stock [g/L]	Stock / final [mL/L]	molar mass [mol]
N	NH_4NO_3	114.29	1	
P	$NaH_2PO_4 * 2H_2O$	50.37	1	0.323
K	K_2SO_4	89.14	1	
Ca	$CaCl2 * 2H_2O$	146.73	1	
Mg	$MgSO_4 * 7H_2O$	405.64	1	
Fe	FeNa - EDTA	15.08	1	
Mn	$MnSO_4 * H_2O$	1.54	1	
Zn	$ZnSO_4 * 7H_2O$	0.04		
Cu	$CuSO_4 * 5 H_2O$	0.04		
Mo	$(NH_4)_6Mo_7O_{24} * 4H_2O$	0.09		
B	H_3BO_3	1.14		

2.4. PRI and SPAD

To determine the photosynthetic activity of the plants the chlorophyll index (SPAD) and the photochemical reflectance index (PRI) were determined on a weekly basis. The measurements were made on the tip, middle and the base of the youngest fully developed leaf. Measuring the base of the leaf is sometimes influence by the morphology of the leaf because the SPAD meter is only able to measure on flat surfaces. SPAD-502 chlorophyll meter (Konica Minolta Sensing Inc., Osaka, Japan) uses two light-emitting diodes (650 and 940 nm) and a photodiode detector to sequentially measure transmission through leaves of red and infrared light.

To adjust the SPAD values for changes in leaf thickness the SPAD values were multiplied with the specific leaf area (SLA) values. Peng et al. (1993) adjusted the SPAD values with SLW because the SPAD meter does not determine leaf thickness.

The PRI values are determined with the PlantPen PRI 200 (Photon Systems Instruments Ltd., Brno, Czech Republic). The PlantPen model PRI 200 measures the photochemical reflectance index in two narrow wavelength bands centered close to 531 nm and 570 nm. PRI is sensitive to changes in carotenoid pigments that are indicative of photosynthetically active light use efficiency, the rate of carbon dioxide uptake, or as a reliable water-stress index. As such, it is used in studies of vegetation productivity and stress according to the manufacturer Photon Systems Instruments. PRI is defined by the following equation using reflectance (ρ) at 531 and 570 nm wavelength.

$$PRI = \frac{p531 - p570}{p531 + p570}$$

Equation 1: Calculation of photochemical reflectance index with wavelengths at 532 and 570 nm with values ranging from -1 to +1.

The results of the PRI PlantPen can be multiplied by 10 or 100 automatically and in this experiment the setting for the “*10” was used. When the results are compared to other experiments like Shrestha et al. (2012) the scale of PRI values is important. Both instruments were calibrated before weekly measurements and during measurements in the VPD chambers calibration was repeated due to the high moisture in the low VPD chamber.

2.5. Biomass, leaf area and specific leaf area

After the last SPAD/PRI measurement the leaf area was determined with a portable area meter (LI-COR Type LI-3000C). The leaf, stem and root biomass was put into paper bags, weighed immediately and then dried in an oven at 60°C for one week and then weighed again with a Kern KB 2400-2N scale for dry matter. The specific leaf (SLA) is calculated with the leaf area [cm^2] divided by the dry matter weight [g] of the leaves.

2.6. Tillers

The tillers were counted on a weekly basis during the measurements of the SPAD/PRI values. In the results the average number of tillers of each treatment is given at the end of each experiment when the leaf area and dry matter were determined.

2.7. P concentration of leaves

Dry leaves were cut into small pieces and milled to fine powder with a steel-ball-mill. Phosphorus content of the leaves was determined after the protocol “Bestimmung von Mineralstoffen in Pflanzensubstanz” from Gericke and Kurmies (1952). The following changes were made compared to the protocol: 0.2 mg dry matter of milled leaf material was used and therefore only half of the chemical liquids were needed. Before the phosphorus content was measured with a Hitachi Spectrometer Type U-3300 the solution was diluted 1:10.

2.8. Statistical analysis and figures

The statistical analysis was done with R-Studio version 3.3.1 (2016-06-21) and for the analysis Multi-way ANOVA to consider two factors (Pstat, Temp) and the responsible variable (leaf area for example). To create the figures from the collected data the SigmaPlot 12.5 is used. To examine the difference between the P-treatments at one specific RZT and VPD of one variety special statistical tools are needed because of the design of the experiment. To identify significant differences at selected cases (e.g. Figure 21 B) an ANOVA was used. The statistical results are displayed in tables in the annex and described under the figures in the results part. The significance level in this thesis is always the Pr (>F) 0.05.

3. Results

3.1. leaf area at different root zone temperatures

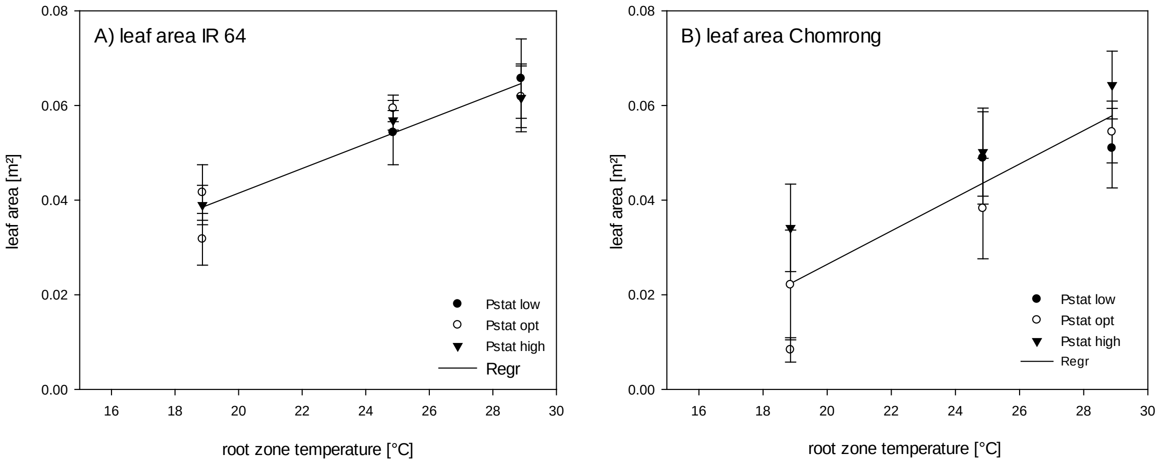

Figure 8: leaf area [m²] of IR64(A) and Chomrong (B) at three different root zone temperatures and P-treatments.

In Figure 8 A) the leaf area of the variety IR64 increased with increasing root zone temperature (RZT). Highly significant difference is seen from the low to high RZT treatment and from the low to medium RZT treatment. There is no significant difference between the medium to high RZT. With all three temperature treatments, the values for the phosphorus (P) treatment are very close considering the scale of the graph.

The variety Chomrong shows a linear increase of leaf area when all values are connected. Sole exception is the value for the phosphorus level low at the medium RZT. Compared to the variety IR64 the standard errors for the average values of Chomrong are wider and the treatments with the highest phosphorus content also have the highest leaf area. There are the same significant differences for the factor RZT as for the variety IR64. From low to high and from medium to low there is a significant difference.

3.2. Dry matter of leaves at different root zone temperatures

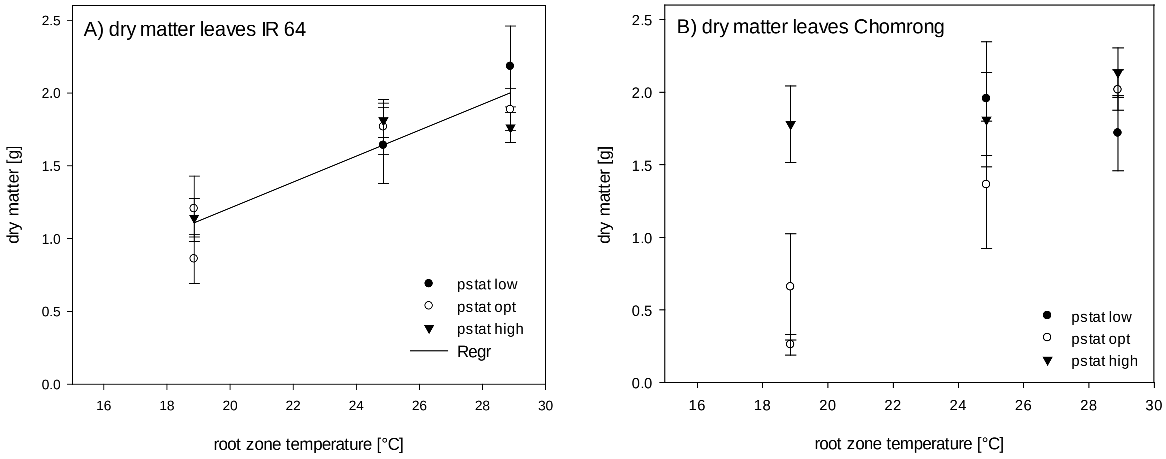

Figure 9: Dry matter weight of the leaves [g] of IR64 (A) and Chomrong (B) at three different root zone temperatures and P-treatments.

Due to no interaction between the RZT and the different P level in the nutrient solution (Pstat) only the root zone temperature for the varieties IR64 and Chomrong were significant different. The dry matter of the IR64 leaves increased with increasing RZT in linear fashion showing a significant difference between the treatment low RZT (18.86°C) and the medium(24.86°C), high(28.89°C) temperature. There is no significant difference between the medium and high RZTtreatment. As seen in the Figure 9 there is no significant difference between the P treatments at three temperature treatments. The dry matter of the leaves is not increased by increasing the concentration of phosphorus in the nutrient solution.

As with IR64 only the RZT has a significant impact on the leaves dry matter weight of the variety Chomrong. Significant differences were found from the low to high RZT treatment and medium to low RZT treatment. The difference between the medium to high treatment is not significant. At the low RZT, the different phosphorus treatments look significant different with the 150% phosphorus content solution the highest dry matter followed by the optimal phosphors treatment. When the low and medium RZT are compared, it is visible that the high P-treatments are on the same level. Providing additional P to the optimal P amount at low root zone temperature increased the dry matter of leaves. With the statistical methods used to evaluate these results there is no possibility to show if there is a significant difference between the P treatments at the low root zone temperature.

3.3. Specific leaf area at different root zone temperatures

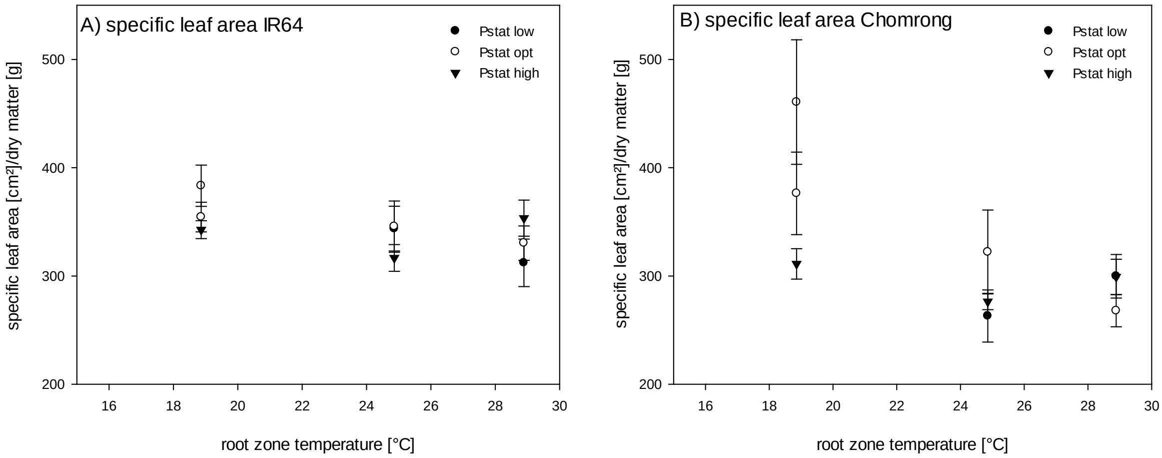

Figure 10: Specific leaf area[cm² g^{-1}] of IR64 (A) and Chomrong (B) at three different root zone temperatures and P-treatments.

The specific leaf area (SLA) of IR64 is not significantly influenced by either RZT or amount of P in the nutrient solution. At the low RZT (~19°C) the SLA is slightly elevated compared to the values at medium and high RZT but the statistical analysis shows no difference at all. For Chomrong the RZT is significantly influencing the SLA. When the values for each temperature are averaged the low root zone temperature is significant different from the medium and high RZT. The highest SLA value by far is the low P-treatment at the low root zone temperature. In the low RZT treatment the SLA values are ordered corresponding to their P-amount with the highest P-treatment the lowest value in this temperature treatment. The high P-treatment of the low RZT treatment is more in line with the values from the medium and high temperature treatments.

3.3 Phosphorus concentration in leaves at different root zone temperatures

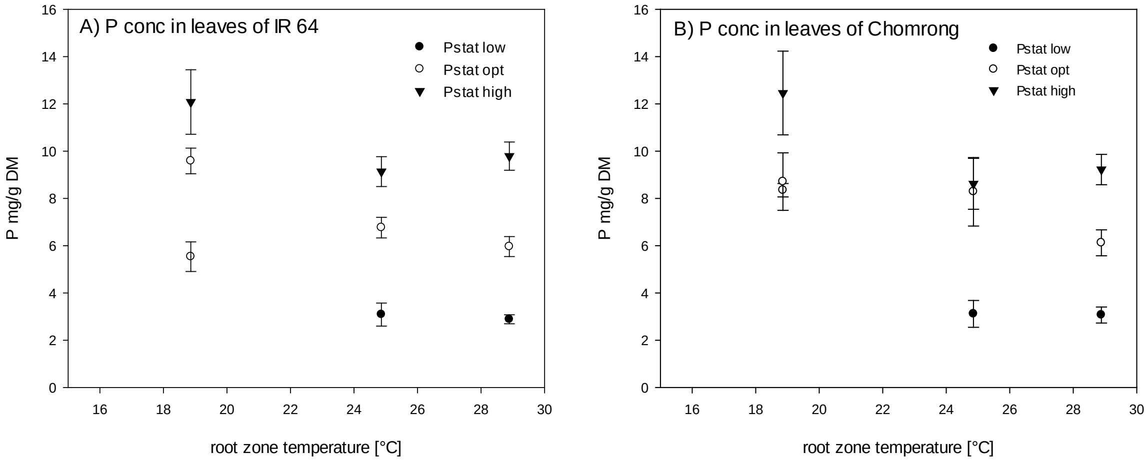

Figure 11: Phosphorus concentration [P mg/g DM] in leaves of A) IR64 and B) Chomrong under three different root zone temperatures and P-treatments.

Three different P treatments are reflected in the results of the phosphorus concentration given in P mg/g dry matter. In figure 11 A) for the variety IR64 we see that there are differences between the different P treatments. The statistical analysis shows that the factor Pstat and the factor RZT have a significant effect on the P concentration in the leaves. For the factor root zone temperature, there was a highly significant difference between the low and high RZT and between the medium to low RZT. The differences between the medium and high RZT are not significantly different. It is remarkable that for the variety IR64 at the low root zone temperature the values for the phosphorus concentration in the leaves are the highest at all phosphorus treatments. The statistical analysis for the factor phosphorus status shows that all differences between the treatments low, medium and high are highly significant.

As with the variety IR64 the factor Pstat and RZT have a significant effect on the P concentration in the leaves of Chomrong. There is no significant interaction between the factor Pstat and RZT. Between all P levels (low, medium and high) there are significant differences. For the factor RZT average values show that there are significant differences between the low to high and medium to low RZT. As with the variety IR64 there is no significant difference between the medium and the high RZT. At the high root zone temperature, the phosphors treatments are in order according to their amount of phosphorus in the nutrient solution with the highest values for the high P treatment.

3.4. P content in leaves at different root zone temperatures

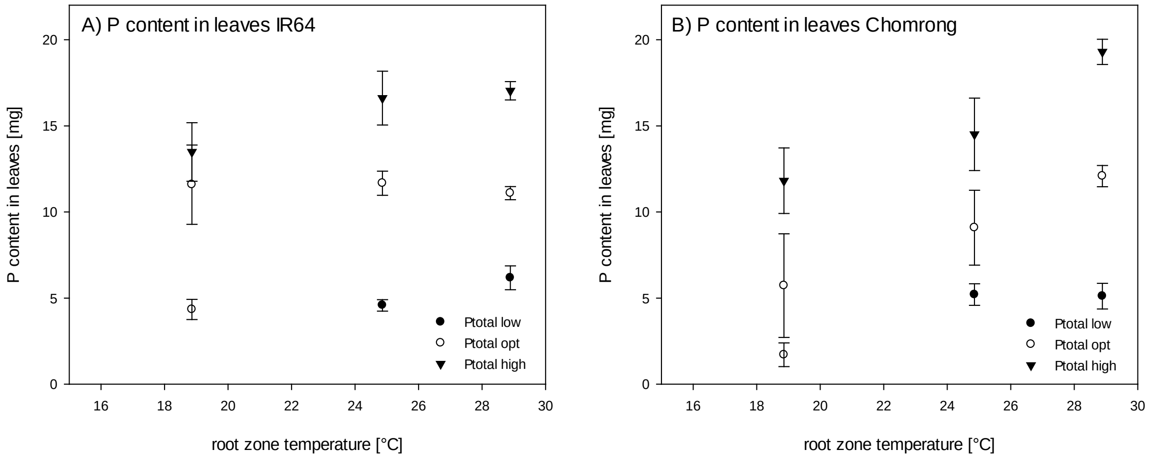

Figure 12: Phosphorus content in leaves [mg] of IR64 (A) and Chomrong (B) under three different root zone temperatures and P-treatments.

To compare the concentration of P with the total amount of P in the leaves of IR64 and Chomrong the concentration [mg/g] is multiplied with the dry matter [g] leaves. The statistical analysis shows that the factor Pstat has a significant effect on the total amount of P in the leaves. There is a significant difference between all the P levels (low, medium and high) for both varieties. Additionally, to the effect of the factor Pstat the factor temperature (Temp) is also significant but only at the variety Chomrong.

The significant difference is between the RZT low to high. No difference is found between the medium to high or medium to low RZT.

3.5. Number of tillers at different root zone temperatures

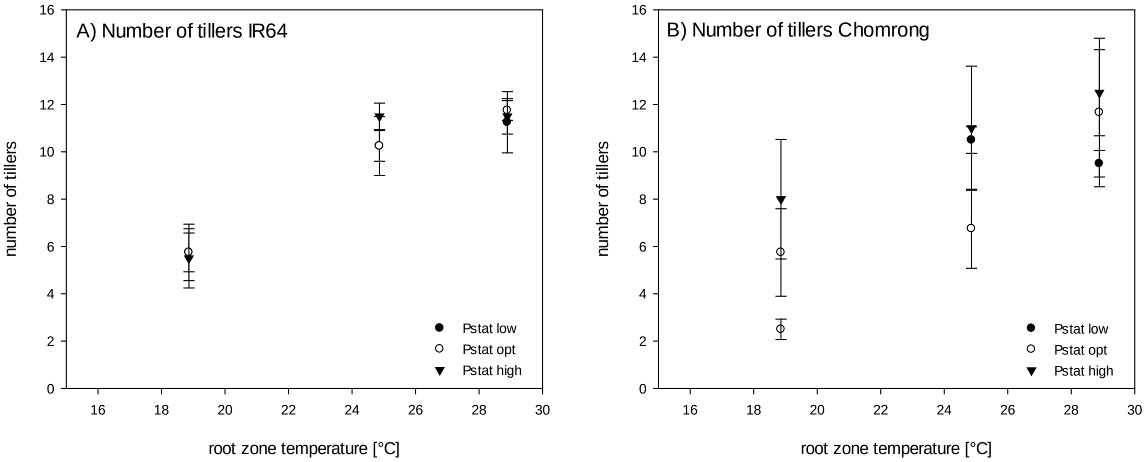

Figure 13: Number of tillers of IR64 A) and Chomrong B) under three different root zone temperatures and P-treatments.

RZT has a significant effect on the number of tillers compared to the amount of phosphorus supplied. The statistical analysis shows that for both varieties the RZT is significantly influencing the number of tillers. In this analysis, the amount of P is not significantly affecting the number of tillers. For the variety IR64 there is significant difference between the low to medium and low to high RZT. There is no significant difference between the medium and high RZT. The values and their standard errors for the different P treatments of each RZT are in close range of each other or are overlapping.

For the variety Chomrong the number of tillers at the RZT low to high and medium low are significantly different. As with IR64 there is no different from the medium to high RZT. Although there is no significant effect of the P at the low RZT of Chomrong there is a visual effect of an increased P amount in the nutrient solution on the number of tillers. With an increased amount of P from 50%, 100% to 150% of the Yoshida EDTA solution there is an increase of tillers from 2.5, 5.75 to 8.0 respectively. In every RZT treatment, the high P with 150% P has the highest number of tillers followed by the optimal P treatment. One exception is at the medium root zone temperature where the low P treatment has the second highest number of tillers right below the high P treatment.

3.6. Photochemical Reflectance Index and SPAD

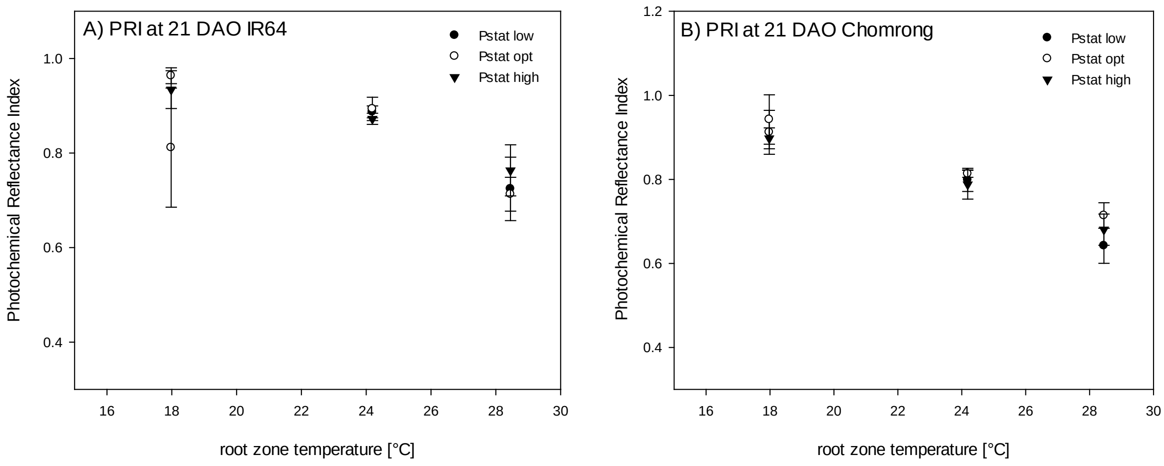

Figure 14: PRI measurement at 21 DAO IR64 (A) and Chomrong (B) under three different root zone temperatures and P-treatments.

The photochemical reflectance index (PRI) is decreasing at the measurement at 21 days after onset of the RZT treatment with increasing temperature from ~18°C up to 28.45°C. For IR64 and Chomrong the factor RZT has a significant effect on the PRI value of the plants. There is a significant difference between the PRI values at the low to high and medium to high RZT. No significant difference was found between the medium to low RZT at IR64. At Chomrong there is a significant difference between all RZT treatments. The factor Pstat has no effect in both varieties on the PRI values.

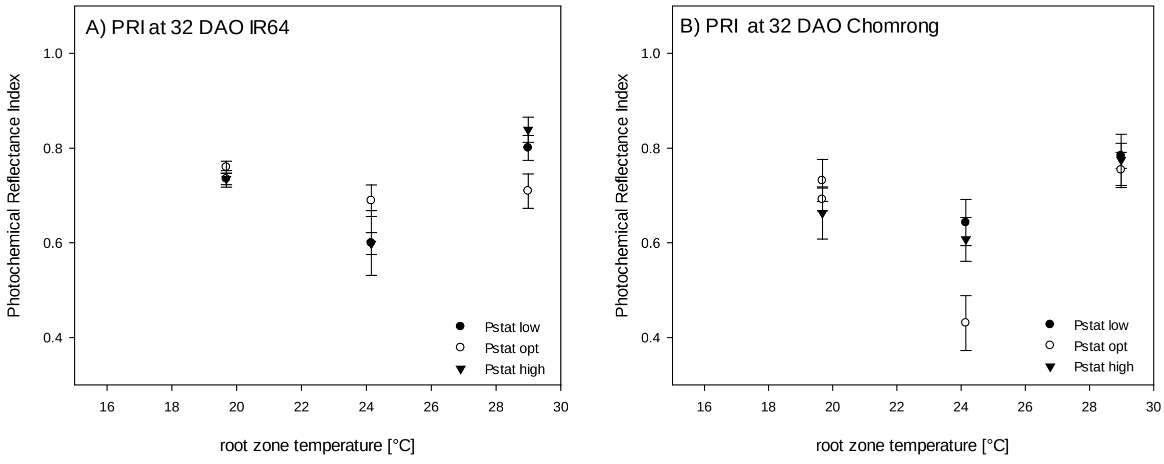

Figure 15: PRI measurement at 32 DAO IR64 (A) and Chomrong (B) under three different root zone temperatures and P-treatments.

This PRI measurement was done 32 days after onset of RZT treatment. In both varieties, the factor RZT has a significant effect on the PRI. In IR64 and in Chomrong the PRI values at the medium RZT are significant different to the low and medium RZT. In Chomrong the control (Pstat opt) at the medium temperature has the lowest value overall and inside this treatment.

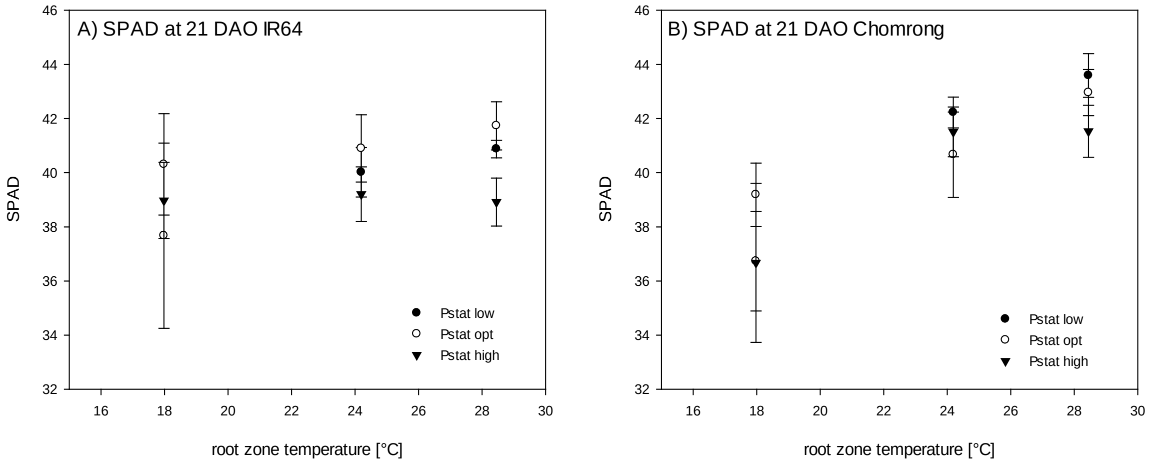

Figure 16: SPAD measurement at 21 DAO IR64 (A) and Chomrong (B) under three different root zone temperatures and P-treatments.

Like the PRI measurement the SPAD measurement was done 21 days after onset of RZT treatment. The SPAD values for the three temperature and P treatments are not significant different and neither the factor temperature nor the factor Pstat influences the SPAD values. In contrast to IR64 the factor RZT at Chomrong influences the SPAD values. The SPAD values at the low RZT differ significantly from those at the medium and high RZT treatment but there is no difference between the medium and high RZT. The factor Pstat and the different P-treatments have no effect on the SPAD values.

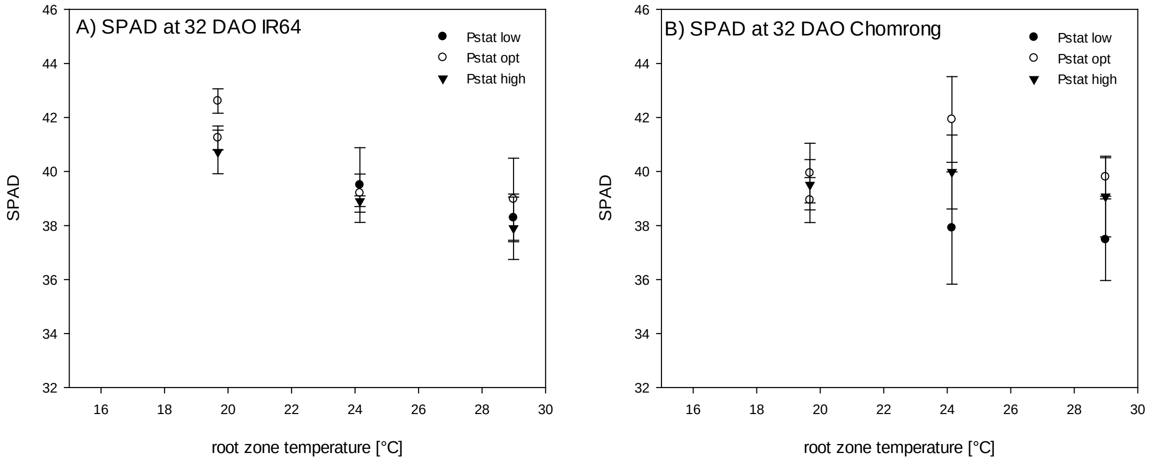

Figure 17: SPAD measurement at 32 DAO IR64 (A) and Chomrong (B) under three different root zone temperatures and P-treatments.

After 32 days after the temperature was changed for the three RZT the SPAD values were measured. The factor temperature is significantly affecting the SPAD values and there are significant differences between the values at the low to medium and low to high RZT. No significant difference is found between the medium (24.15°C) and the high (28.99°C) RZT. Both factors temperature and Pstat are not significant for the variety Chomrong in this experiment. It is remarkable that at the medium (24.15°C) treatment the standard errors are quiet high compared to the low RZT of Chomrong.

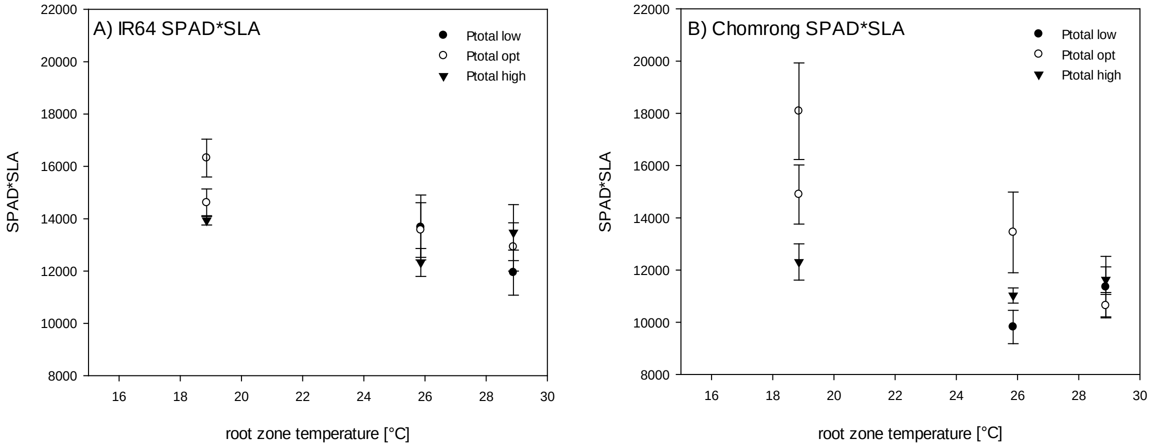

Figure 18: Adjusted values of SPAD with SLA of IR64 (A) and Chomrong (B) under three different root zone temperatures and P-treatments.

In IR64 the root zone temperature is the factor affecting the SPAD*SLA values and there is a significant difference between the low and high RZT. The medium RZT is not significantly different from the low and high RZT. The factor Pstat has no effect and there is no significant difference between the P-treatments.

The factor temperature has significant effect on the SPAD*SLA values and there is also a significant interaction between P status and temperature in Chomrong. The SPAD*SLA values at the low RZT are significant different to the values from the medium and high RZT treatment. At the low RZT the low P-treatment has the highest value followed by the optimal and high P-treatment respectively.

3.8. Leaf area under varying VPD

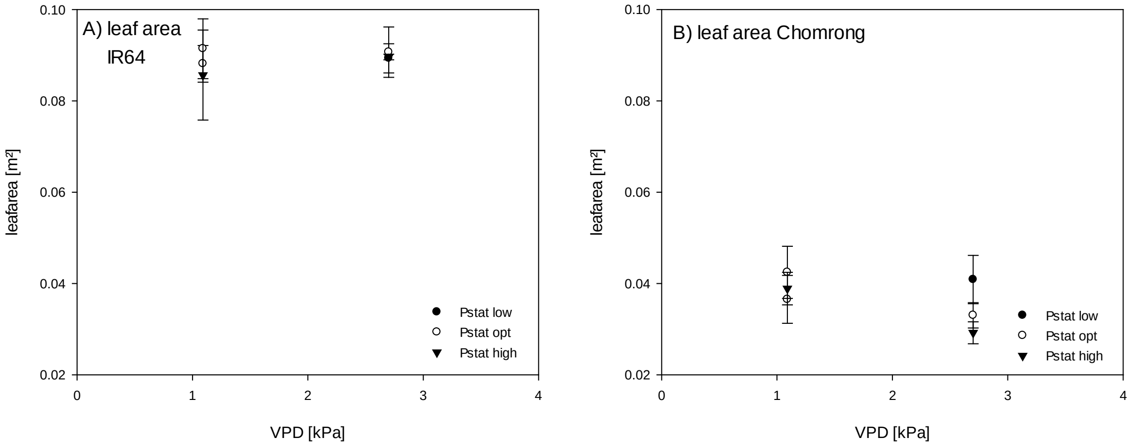

Figure 19: Leaf area [m²] of IR64 (A) and Chomrong (B) under different VPD

Under different vapor pressure deficit (VPD) conditions at low (1.09 kPA) and high (2.70 kPA) the leaf area for IR64 and Chomrong are not significant different. The factors VPD and Pstat have no effect on the leaf area and are not significant. The variety IR64 has significant more leaf area [m²] compared to the variety Chomrong under low and high VPD. The average air temperature under low VPD and high VPD were 31,98°C and 31.91°C respectively. The amount of P in the nutrient solution does not affect the leaf area at all three P-treatments.

3.6. Dry matter leaves, dry matter plant and specific leaf area under varying VPD

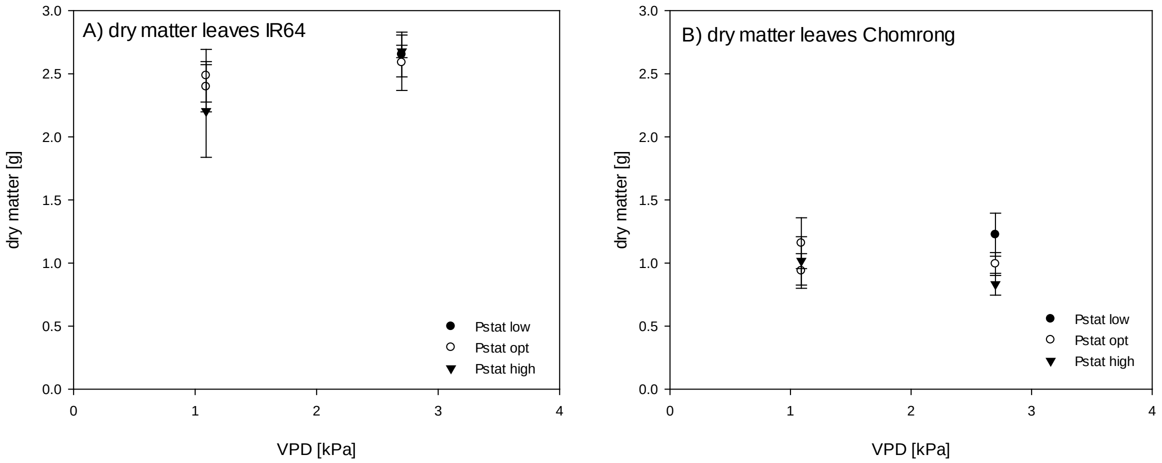

Figure 20: Leave dry matter weight [g] of IR64 (A)and Chomrong (B)under different VPD.

The dry matter weight of the leaves was determined for the varieties IR64 and Chomrong under low (1.09 kPA) and high (2.70 kPa) VPD. Figure 20 shows that there are slightly higher values for the high VPD treatment but there are not significant different to the low VPD treatment in both varieties.

In addition, there is no significant difference between the P treatments and the low VPD shows higher standard errors compared to high VPD treatment. Additional P in the nutrient solution has no significant effect on the dry matter of variety IR64.

Compared to variety IR64 the variety Chomrong has a lower dry matter weight in both treatments but there is also no significant influence of the P concentration in the nutrient solution and the VPD level on the dry matter. Increasing the supply ofP to the Chomrong plants in the Pstat high treatments has no effect and these treatments had the second lowest value in the treatment low VPD. In the high VPD treatment the Pstat high with 150% P has the lowest dry matter content of all treatments.

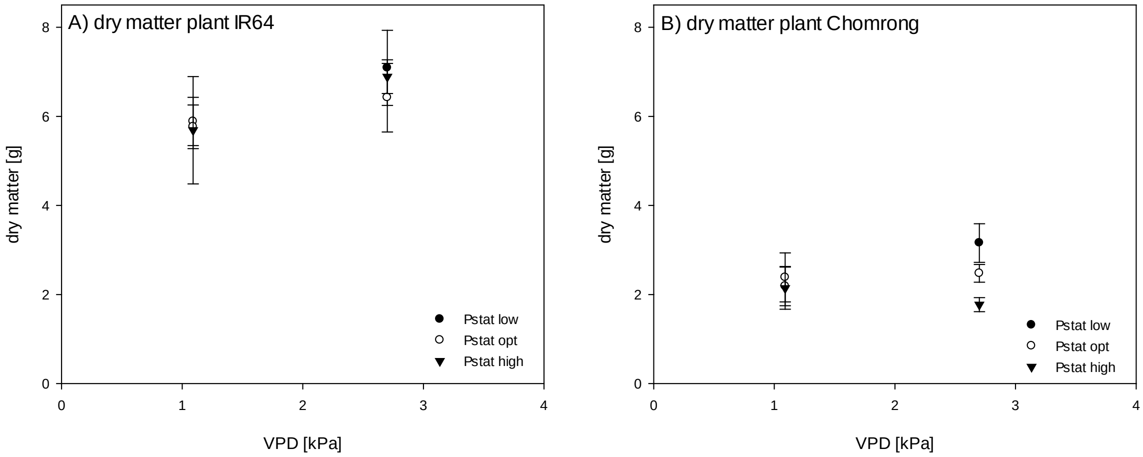

Figure 21: Dry matter weight of plants [g] IR64 (A) and Chomrong (B) under different VPD.

Additional to the leaf dry matter weight also the whole plant dry matter as the sum of roots, stems and leaves were determined. In Figure 21 A) the variety IR64 displays greater standard errors compared to the variety Chomrong (Figure 21 B) although the values for IR64 for both VPD treatments are near of each other. The statistical analysis shows that for both varieties that there is no significant influence of the factors phosphorus and VPD level. Compared to the variety IR64 the variety Chomrong has a remarkable order of the different P levels at the high VPD treatment. In this treatment, the lowest phosphorus treatment has the highest plant dry matter weight and the highest P treatment has the lowest plant dry weight.

The statistical analysis with an ANOVA of high VPD treatment of Chomrong shows that the low Pstat is significant different to the high P-treatment. No significant difference is found between the optimal and high P-treatment.

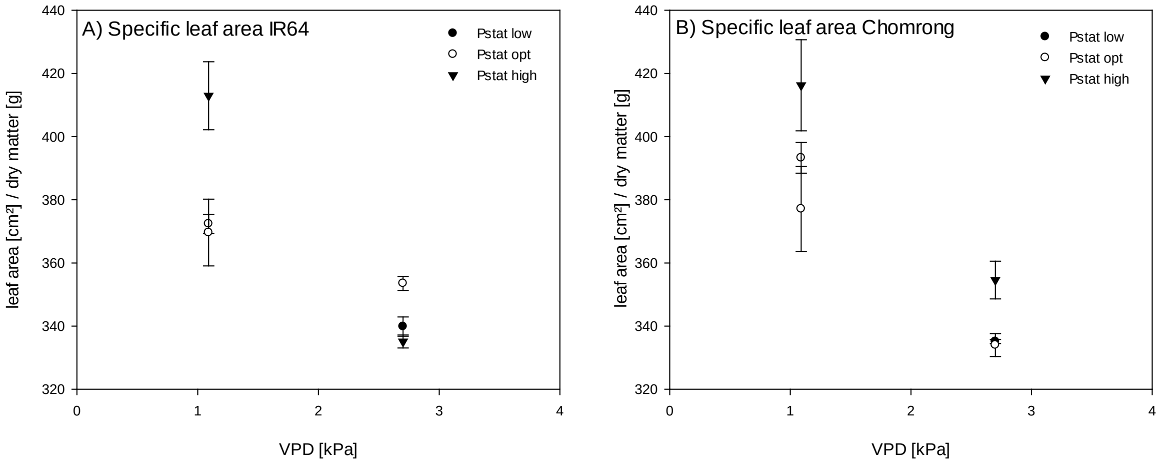

Figure 22: Specific leaf area [cm²/g] A) of IR64 (A) and Chomrong (B) under varying VPD.

The SLA [cm²/dry matter g] is significantly higher at the low VPD level compared to the high VPD level in both varieties. In Chomrong the high P-treatment has the highest SLA value at the low and high VPD level compared to IR64 where only at the low VPD level the high Pstat has by far the highest value for SLA. At the high VPD level of IR64 the high Pstat has the lowest value. This is interesting compared to Chomrong at the same level and other VPD treatment.

3.9. P concentration in leaves of IR64 and Chomrong

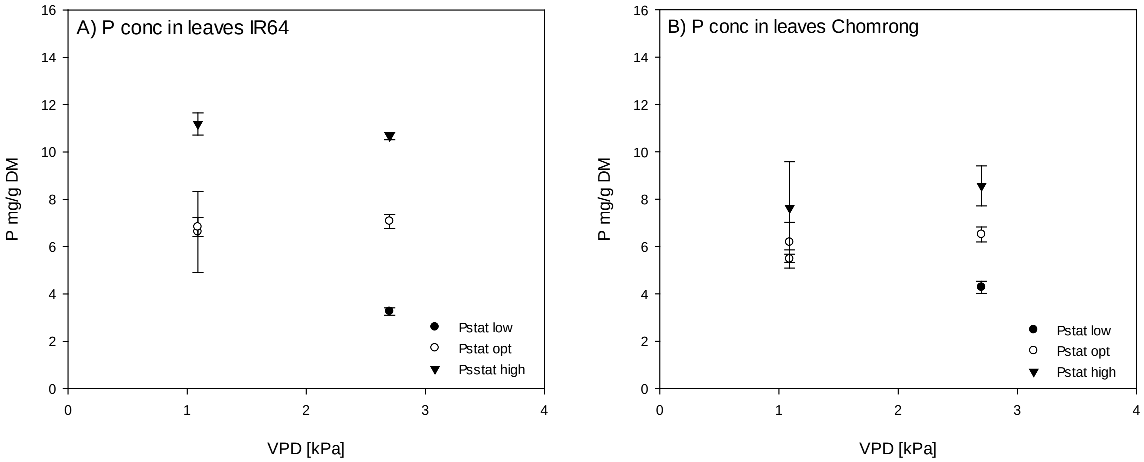

Figure 23: Phosphorus concentration in leaves of IR64 (A) and Chomrong (B) under different VPD.

The concentration of P inside the leaves of IR64 is significantly influenced by the concentration of P inside the nutrient solution. For the variety IR64 there are significant differences between the low to high and medium to high P concentration inside the nutrient solution. The values for the high VPD treatment show small standard errors and are ordered corresponding to their amount of P in the nutrient solution with the highest value for the Pstat high. In contrast to the high VPD treatment the low VPD treatment shows high standard error for the Pstat low (1.708)and the average values for the Pstat opt and Pstat low treatments of IR64 are very close. The pattern seen in variety IR64 can also be seen in variety Chomrong but in a smaller scale.

At the low VPD treatment of Chomrong higher standard errors are displayed at the Pstat high (1.952) and Pstat opt (0.845) compared to the high VPD treatment. As with IR64 the high VPD treatment of Chomrong is ordered corresponding the P concentration inside the nutrient solution. The statistical analysis shows that there is a significant effect of the P nutrition and there is significant difference between the low and high P treatment in Chomrong. In every treatment of VPD and in both varieties, the plants with the high P concentration inside the nutrient solution also have the highest P concentration in the leaves.

3.10. P content in leaves of IR64 and Chomrong

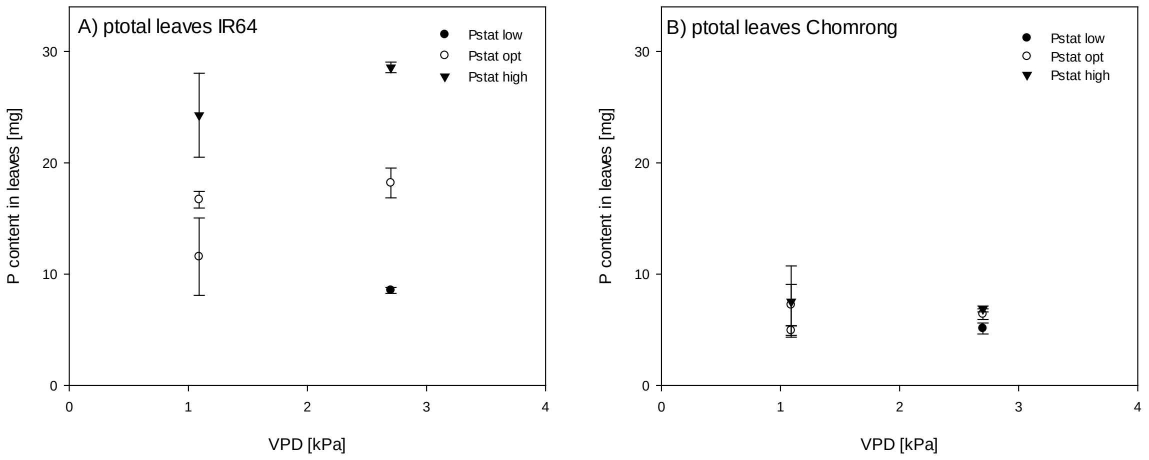

Figure 24: Phosphorus content in leaves of IR64 (A) and Chomrong (B) under varying VPD.

P content is the result of P concentration and dry matter of the leaves. The factor Pstat has a significant effect on the P content in the leaves of IR64. There are significant differences between the low, medium and high Pstat but there is no effect of VPD. In Chomrong there is no effect of increased P and VPD on the P content.

3.11. Number of tillers of IR64 and Chomrong under varying VPD

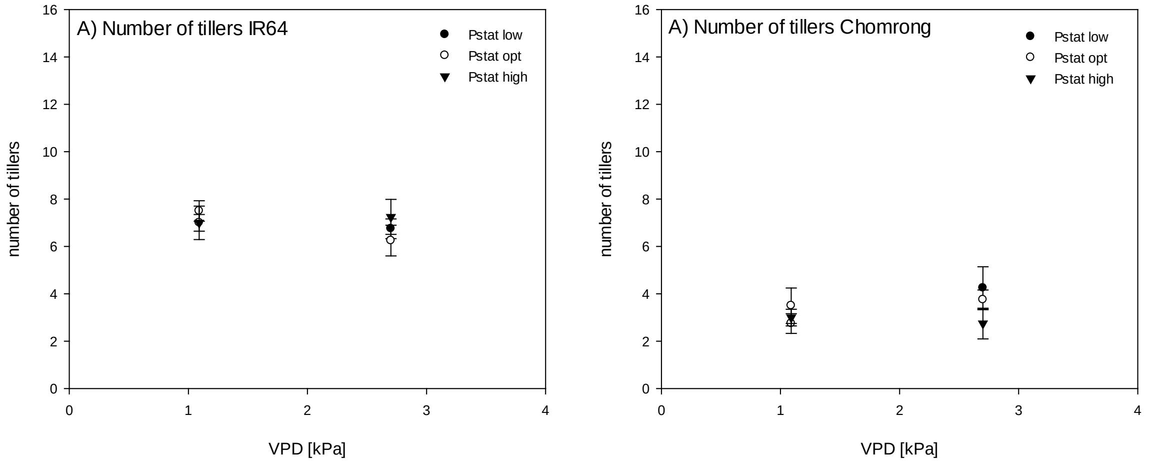

Figure 25: Number of tillers of IR64(A) and Chomrong (B) under varying VPD.

Different VPD environments with high and low VPD have no effect on the number of tillers neither does the amount of P in the nutrient solution. The statistical analysis shows that there is no significant effect of the factors P or VPD level on the tillering characteristics of both varieties IR64 and Chomrong. The variety Chomrong develops less tillers in the VPD chambers compared to IR64 under the same conditions.

3.11. Photochemical Reflectance Index and SPAD

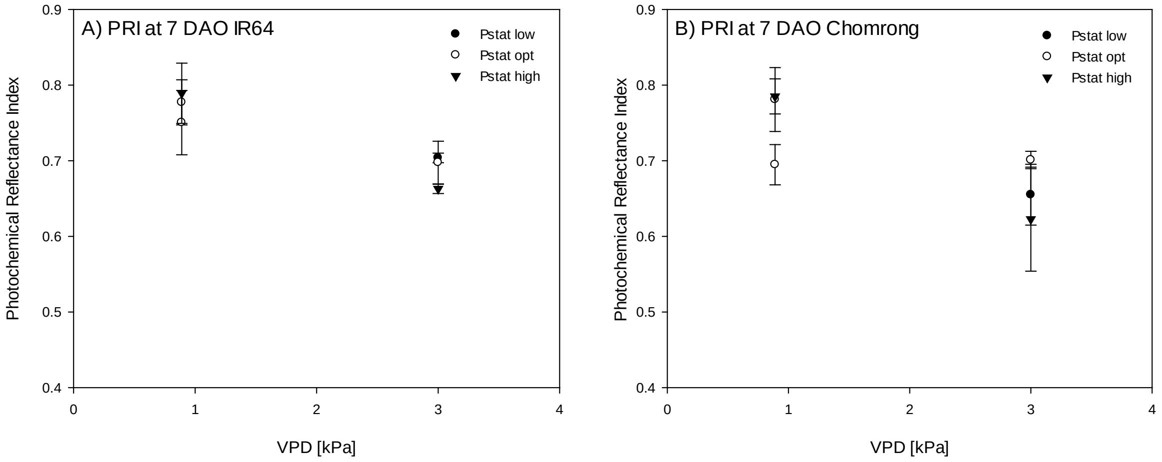

Figure 26: PRI measurement at 7 DAO of IR64 (A) and Chomrong (B) under different VPD.

The results for the PRI measurement after 7 days after onset of treatment (DAO) is shown in figure 26. Significant difference is found between the low (0.89 kPa) and high (3.00 kPa) VPD level in both IR64 and Chomrong. In contrast to the factor VPD the factor Pstat is not affecting the PRI values. There is no significant difference between the two varieties at the beginning (7 DAO) of this experiment.

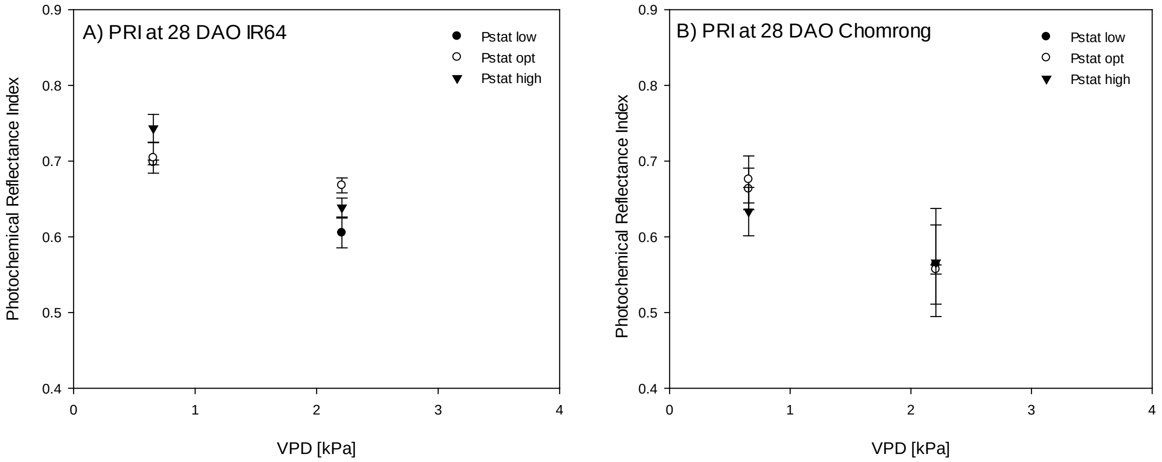

Figure 27: PRI measurement at 28 DAO at IR64 (A) and Chomrong (B) under different VPD.

After 28 DAO, another measurement for PRI is done and like in the previous measurements there is a significant difference in both varieties between the low (0.66 kPa) and the high (2.21 kPa) VPD level. In addition to the significant difference between the VPD levels there is also a significant difference between IR64 and Chomrong with IR64 having significant higher PRI values at both VPD levels.

The measurement at 28 DAO is chosen because it is at the end of the experiment and therefore the adaptation to the environment with high and low humidity of the individual variety can be shown and comparison to the measurement at 7 DAO can be made.

The Fig 28 (Annex) shows the 5 measurements from 1 DAO to 28 DAO at the low and high VPD for IR64 and Chomrong. In this graph the reduction of PRI over the time can be seen in all varieties and treatments and the lower values of PRI at high VPD. When the graphs for low and high VPD are compared, it is noticeable that the reduction is the same in both treatments but the starting point for the low VPD is higher compared to the high VPD. There are also differences between the varieties when the development of values over time is compared. IR64 has a rather smooth reduction in contrast to Chomrong where there is also an increase at 21 DAO followed by a reduction to 28 DAO. Further measurement is needed to prove a trend in the reduction of PRI values in the long term.

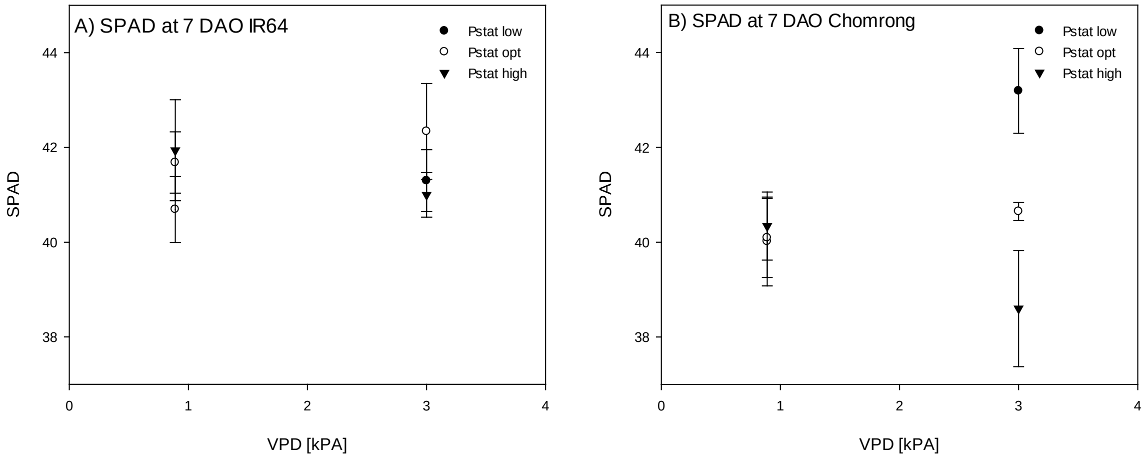

Figure 28: SPAD measurement at 7 DAO of IR64 (A) and Chomrong (B) under different VPD.

The statistical analysis showed that there is no significant effect of the P concentration in the nutrient solution nor the level of VPD on the SPAD values for both varieties. Although there is no significant difference based on the averages of values of low and high VPD for the P level it seems that at the high VPD treatment of Chomrong a significant difference between the Pstat.

The figure 30 (Annex) show an increase of SPAD values over the duration of 5 weeks (weekly measurement) beginning from the first measurement at 1 DAO until 28 DAO. In most measurements, there is no difference between the Pstat (low, opt and high) but especially at 14 DAO in the low VPD of IR64 and Chomrong lower values for SPAD and differences between the Pstat with greater standard errors can be seen. Due to five measurements, the measurement at 14 DAO is examined in detailed and compared to the other measurements at 28 DAO.

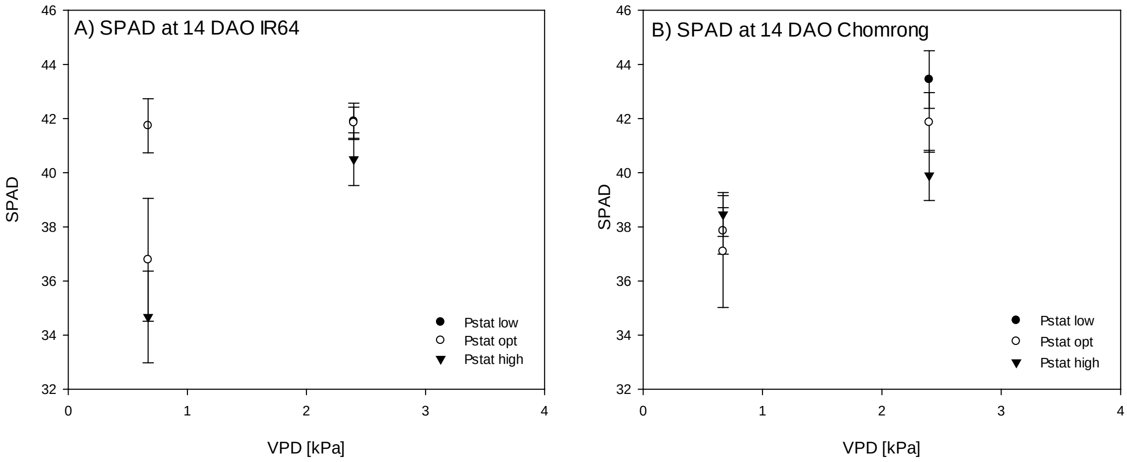

Figure 29: SPAD measurement at14 DAO of IR64 (A) and Chomrong (B) under different VPD.

In the variety IR64 the Pstat and the VPD have significant influence on the SPAD values (Figure 31 A). There is a significant difference between the low and high VPD level and between the low and high Pstat. It is also important to notice that the SPAD values of IR64 at the low VPD level are accompanied with quite high standard errors. In contrast to IR64 Chomrong has a significant difference between the VPD levels. The high (2.40 kPA) VPD have significant higher SPAD values than the low (0.67 kPa) VPD treatment.

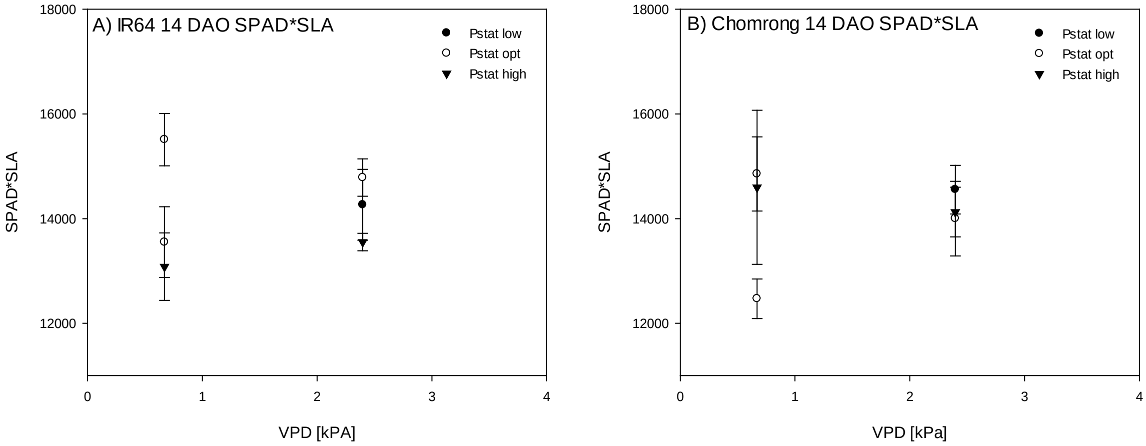

Figure 30: Adjusted values of SPAD with SLA at 14 DAO of IR64 (A) and Chomrong (B) under different VPD.

To take the thickness of the leaves into consideration the SPAD values were multiplied with the SLA values from the final measurement. The analysis shows no significant influence of Pstat or VPD on the SPAD*SLA values. Compared to the low VPD values the high VPD values for both varieties are in a tighter pattern.

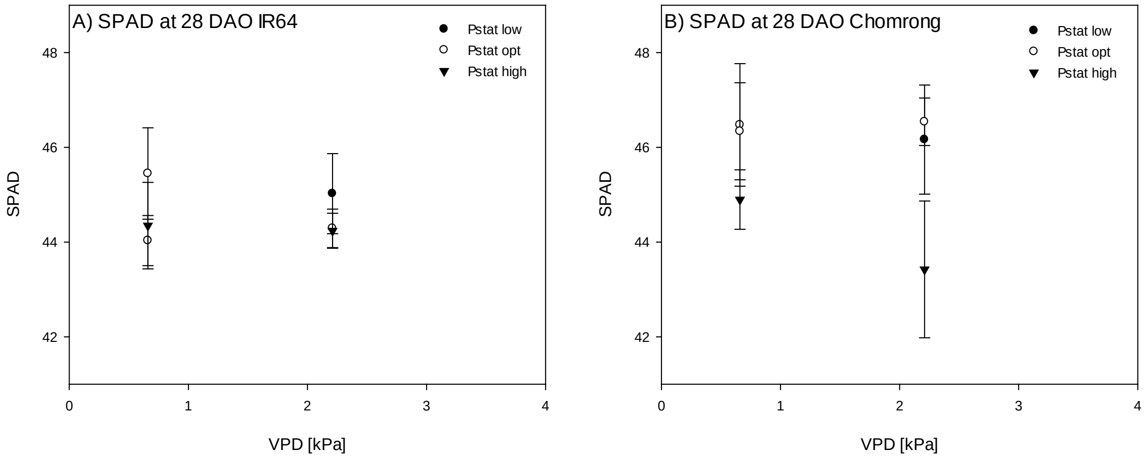

Figure 31: SPAD measurement at 28 DAO of IR64 (A) and Chomrong (B) under different VPD.

The measurement after 28 DAO was the last measurement of SPAD and is therefore at the end of the experiment. The factor Pstat and VPD have no significant effect on the SPAD values in IR64 and Chomrong. Especially in Chomrong at both VPD treatments there are high standard errors.

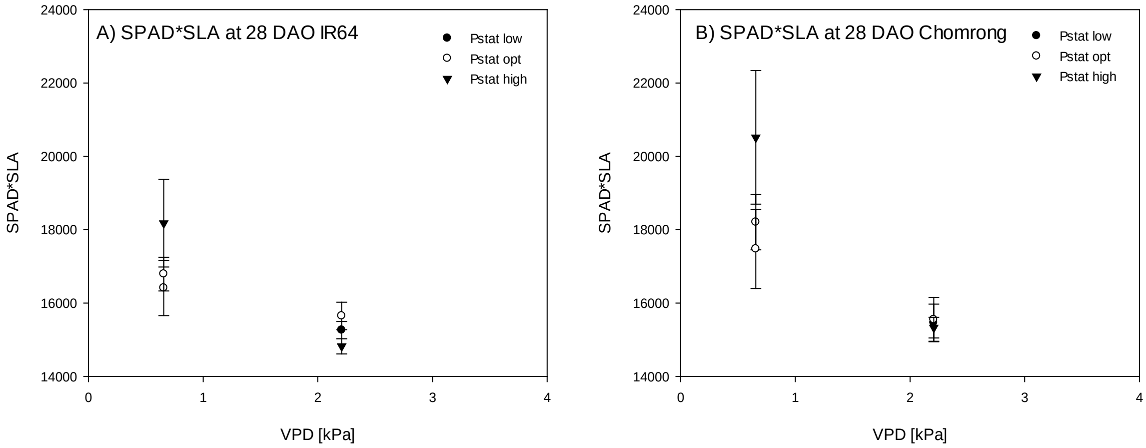

Figure 32: Adjusted values of SPAD with SLA at 28 DAO of IR64 (A) and Chomrong (B) under different VPD.

To adjust for different SLA values (leaf thickness) the SPAD values were multiplied with SLA values just like done in the measurement at 14 DAO. Significant differences are found between the low and high VPD treatments in IR64 and Chomrong. The SPAD*SLA values at the high VPD treatment have significant lower values than the low VPD treatment. The SLA also had a significant difference between the VPD level.

4. Discussion

4.1. Leaf area at different root zone temperatures

Low RZT significantly reduces the leaf area in both varieties IR64 and Chomrong in the pot experiment compared to the higher root zone temperatures in this experiment. According to Sato (1972) leaf growth is sensitive to environmental stresses like low water temperatures and the water temperature in general is influencing the growth of rice strongly in the first half of the growing season. In addition, Shimono et al. (2002) suggest that the effect of low water temperature on the leaf area development is consistent over the years under different radiation and air temperature conditions. In this experiment the low RZT averaging at around 19°C decreased the leaf area for all phosphorus treatments especially in variety IR64. Similar finding for reduced leaf area under low root zone temperature were reported by Shimono et al. (2002) and Nagasuga et al. (2011).

With increasing RZT leaf area is increasing in a linear fashion as seen in figure 8. Similar linear relationship was reported by Shimono et al. (2002) with the relative leaf growth rate (RLGR) and the tested temperature ranging from 15.6°C to 24.6°C. Although it is important to note that the relative leaf growth rate is closely and positively correlated with the tillering and leaf appearance. Leaf area growth is an integrated result of tillering, leaf appearance rate and leaf elongation (Shimono et al. 2002).In contrast to low RZT higher RZT temperatures increased the leaf area in this experiment. The medium RZT averaged at 25°C and the RZT treatment at ~29°C. Ishii et al. (2011) reports that high water temperatures (21°C to 24°C) during the vegetative growth strongly enhanced early growth and is affecting the leaf area positively. In this experiment the leaf area for Chomrong the optimal P concentration increased 0.0027m^2 per °C from the low to high RZT treatment and 0.004m^2 per °C for the medium to high RZT treatment. The leaf area of IR64 increased for 0.003m^2 and 0.0006m^{2}per °C respectively. These results show that there is also a difference between the tested varieties concerning the temperature behavior. According to Ishii et al. (2011) the enhanced growth is a direct result of high temperature on cell division and elongation of individual leaf cells in developing leaves described by Granier and Tardieu (1998) and accelerated appearance of leaves (Takamura et al. 1960). An increased canopy photosynthesis can supply more carbohydrates to young leaves and this might influence the leaf appearance and expansion (Morvan-Bertrand et al. 2001).

The effect of P treatments on the leaf area is more pronounced in the variety Chomrong than the variety IR64 where the values are in close proximity. With Chomrong the highest P treatment has the highest leaf area over every RZT treatment and at the low RZT of Chomrong there is clearly an effect of phosphorus. These results are supported by the findings of Rao and Terry (1995) where the leaf area expansion was reduced at low phosphorus treatment of sugar beets grown in hydroponic system.

The low P-treatment received only the half of the control P-treatment as in this experiment. According to this results there is no effect of P on the leaf area but the increased leaf area at the low temperature treatment show the opposite. The results suggest that with an increased P-fertilization for Chomrong at low RZT at planting time the leaf area is positively affected and therefore also the growth in the vegetative stage. Further work was done by Abadia et al. (1987) in sugar beets where also the leaf area at low phosphorus treatment (50% of control) was reduced by 80% compared to the control. Although this kind of reduction in leaf area where not seen in the low temperature treatment of IR64 the leaf area of low P-treatment is reduced by 23,7% compared to the control. The leaf area of IR64 plants is not increased with more P than 100% P of the control. It seems that the Yoshida EDTA solutions are optimized for such varieties like IR64.With the variety Chomrong at the low temperature treatment the leaf area was reduced by 62% at the low P-treatment and increased by 35% at the high P-treatment. Suggesting that additional P to the control treatment is beneficial.

4.2. Dry matter leaves and specific leaf area at different root zone temperatures

The increasing RZT from 19°C to 29°C also increased the dry matter of the leaves of IR64. For the low P-treatment even a linear increase of dry matter can be described in figure 9. The control P-treatment (Pstat opt) also displays a linear increase when the three temperature treatments are connected. Nagasuga et al. (2011) also reports significant reduction of leaf dry weight in rice plants under low root zone temperature compared to the control temperature. The low root zone temperature in this experiment is 5°C higher than the temperature used by Nagasuga et al. (2011). With 14°C used by Nagasuga et al. (2011) as the lowest temperature treatment a more pronounced difference between the temperature treatments can be expected. The temperature difference of 6°C is enough to show significant differences between the low and medium RZT treatment in this experiment.

The allocation of dry matter to the leaves is significantly reduced by low root RZT but the net assimilation rate (NAR) is not significantly affected. (Nagasuga et al. 2011) This suggests that the low RZT is not affecting the photosynthetic activity, the reduction in dry matter in leaves and the total dry matter of the plant is the effect of a reduced leaf area (Shimono et al. 2002). Although total plant weight was not measured in this experiment the dry matter weight of leaves and total plant dry matter are strongly correlated.

At the low RZT low P-treatment decreased the dry matter of the variety IR64 and especially the variety Chomrong. At the medium RZT the arrangement is not as clear as at the low and high RZT treatments in both varieties. Low P-treatment at chilling (1-5°C) of the whole plant reduced the dry matter distribution to the blades significantly compared to the control treatment which received appropriate amount of P (Starck et al. 2000). Not only the leaves but also the whole plant is affected by low P treatment.

At the low RZT treatment of Chomrong there is a visible difference between the control (Pstat opt) and the low P-treatment. Although the difference is more visible at low RZT in Chomrong the control P-treatment has high values in IR64.

The high P-treatment of Chomrong at the low RZT shows that high P-treatment has the highest leaf dry matter and an increase in P-fertilization is beneficial for the dry matter content of the leaves. Seneweera et al. (1994) did a pot experiment with a range of 0 – 480 mg P kg^{-1} soil and found that stem and leaf weight were increased by higher soil P levels. If low-P treatment at low RZT is reducing the allocation of dry matter to the leaves than an increased P availability might positively influence the allocation to the leaves. Although there is no effect of increased P in the leaves of IR64 the variety Chomrong shows an increased dry matter especially at the low RZT and also a very small increase at the high RZT.

Leaf area [cm^2] and leaf dry matter [g] determines the specific leaf area [cm^2g^{-1}] of the plant under different treatments. According to Nagasuga et al. (2011) the specific leaf area is reduced under low root temperature. In both varieties, the specific leaf area of the controls (Pstat opt) are declining with increasing temperature especially in Chomrong with a steeper slope. The reduction of specific leaf area is also reported by Kuwagata et al. (2012) under the RZT of 13°C compared to 25°C under low air humidity and by Shimono et al. (2002) in the vegetative period over several years. Although in this experiment the specific leaf area is higher in the low temperature treatments. The low P-treatment of Chomrong at the low RZT (19°C) has the a very small leaf area and compared to the other P-treatments a low leaf dry matter but the specific leaf area is the highest. The high SLA values indicates that the low-P Chomrong plants at the low temperature have very small leaves and low dry weight resulting in a high SLA value compared to the optimal or high P-treatment plants at the low root zone temperature. Compared to the low-P Chomrong plants the high-P Chomrong plants have the highest leaf area and dry matter in the low temperature treatment.

It seems that these results from this experiment are not in line with the literature described above. The researchers used different temperatures for the low RZT compared to this experiment. Shimono et al. (2002) set the temperature to about 15°C, Kuwagata et al. (2012) even to 13°C and Nagasuga et al. (2011) to 14.0°C. Additionally to the low RZT the low P-treatment is affecting the SLA when it is compared to the high P-treatment. Although the factor Pstat is not affecting the SLA significantly there is a visual difference between the high P-treatment and the control.

4.3. Phosphorus concentration in the leaves at different root zone temperatures

At the low RZT the concentration of P is increased compared to the higher RZT. In this experiment the P concentration of all P-treatments in the low (19°C) RZT treatment is significant higher in IR64 and Chomrong compared to the higher temperatures at the root zone. Similar results were found by Engels (1993) with wheat were the P concentration increased in the shoot fresh weight at the low RZT of 12°C after 16 days of treatment. The results in this experiments were based on the concentration of P [mg g^{-1}] of the leaf dry matter [g]. In Chomrong the control P-treatment differs significantly but the difference from the low to the medium RZT is not immediately visible.

The low and optimal P-treatment of Chomrong at the low RZT has the same value than the high P-treatment at the medium and high RZT. There might be an improved uptake at low RZT because Chomrong is tolerant to cold environments and is well adapted to yearly sowing in high altitudes (Shrestha et al. 2011). Chomrong shows highest yielding in low yielding environments and low to medium yield in high yielding environments (Shrestha et al. 2011). In contrast to other short-duration rice cultivars where Seneweera et al. (1994) found that with higher P levels the grain number and the yields can be increased.

With increasing P in the nutrient solution also P concentration in the leaves increased for all treatments and both varieties. Same results were found by Zhou et al. (2009) that with increasing P in the nutrient solution not only the P concentration [mg per g DM] in the leaves but also in the shoot increased. Fageria et al. (1982) found in upland rice field trials that P uptake increase with the increasing levels of P and advancing age of the rice crop.

The figure 12 of the P content in the leaves also confirms that with increasing P content in the nutrient solution the total amount of P in the leaves is increasing. Although the low RZT has the highest concentrations of P in every P-treatment compared to the total amount of P they have the lowest in every P-treatment. The small leaf area of Chomrong at the low RZT compared to the higher RZT and the lower dry matter of the leaves leads to a high concentration of P. The total P amount of Chomrong leaves is increasing with increasing P in the nutrient solution and the increase of temperature up to 25°C. Makino et al. (1984) reported that amount of P in the leaf was the lowest compared to other nutrients when the respective nutrient (P) was not in the nutrient solution. In this experiment the low P-treatment is 50% of the control but the same effect described by Makino et al. (1984) can be seen in figure 12. The low P-treatment in every RZT and in both varieties has the lowest value and the total amount of P is increased with increasing P in the nutrient solution. Also Engels (1993) reported that the net uptake rates of P are decreased with decreasing RZT in wheat. To achieve similar results the concentration of P must be significantly lower in the low RZT of this experiment. It could be that the 19°C as low RZT is not too low to decrease the uptake of P. The root zone temperatures used by Engels (1993) were 12°C,16°C and 20°C and therefore any comparison is tough to make.

Mainly the reduced leaf area and dry matter weight as described above are the major reason for the lower total P content of the leaves in the low RZT treatment.

4.4. Number of tillers at different root zone temperatures

Low RZT decreased the number of tillers and therefore higher root zone temperature should increase the number of tillers. Takamura et al. (1960) found that low RZT is reducing the tillering rate, leaf appearance and leaf elongation. According to Shimono et al. (2002) the relative crop growth rate is closely and positively correlated with the relative tillering rate and the leaf appearance rate. This correlation shows that the limiting effect of low RZT on the leaf area development is caused by both processes. The delay and reduced rate of tillering due to low RZT is also reported by Shimono et al. (2007). In this experiment the low RZT decreased the numbers of tillers significantly in the varieties IR64 and Chomrong.

In contrast to the low RZT higher RZT should increase the number of tillers but the high temperature treatment (29°C) does not increase the number of tillers significantly compared with the medium RZT (25°C). Although high water temperature 24.1 ± 2.9°C increased vegetative growth in early stage Ishii et al. (2011) reported no significant increase in tiller production to the control temperature (21.3 ± 3.0°C). Same results were obtained in this experiment were the medium RZT (25°C) has no significant difference in tiller numbers to the high RZT (29°C). Ishii et al. (2011) also reported that 21-24°C might be within the optimal temperature range of the variety “Hitomebore”. Like the variety tested by Ishii et al. (2011) an increase in RZT does not have a significant effect on the tiller numbers of the varieties IR64 and Chomrong. Matsushima et al. (1964) reports that a temperature of 21°C for air and water temperature is the optimum for increasing the tiller number in lowland rice seedlings. In this experiment the average air temperature in the greenhouse was at 31.77°C. According to the experiments of Matsushima et al. (1964) at 31°C air temperature and 21°C water temperature 65% of theoretical tiller appeared and with an increase of the water temperature to 31°C only 35% of the tillers appeared.

An increased amount of P in the nutrient solution is increasing the number of tillers in Chomrong especially under low RZT. Fageria et al. (1982) reported an increase of tillers/m² in upland rice with increasing amount of P-fertilizer in upland rice. The results of a study to screen 25 rice cultivars at low, medium and high soil-P showed that the number of tillers were related to tissue P concentration and the number of tillers increased with increasing P-fertilization. It is important to note that there are significant cultivar differences between cultivars for tillers, P concentration in shoots and P content of shoots (Fageria et al. 1988). The results of this experiment are therefore in line with the reported increase of tiller with increase of P. When the two varieties are compared concerning their response to increase P-concentration in their nutrient solution IR64 shows little to no response to additional P. Chomrong on the other hand has higher degree of standard errors but also responses to increased P especially in the low RZT. The response of IR64 can be described as temperature depended while Chomrong responses nicely to increased P-fertilization. Although it is reported by Chang and Vergara (1975) that rice plants under upland conditions have fewer tillers and leaf area. In this experiment under average air temperature of 31.77°C and up to 29°C water temperature the upland variety Chomrong responds well concerning tiller number when compared to lowland variety IR64 (Shrestha et al. 2011).

4.5. Photochemical Reflectance Index

The high PRI values indicate that the plants 21 DAO at the low RZT dissipate more excess light energy mediated by the xanthophyll cycle according to Guo and Trotter (2006). These xanthophyll cycle pigments are closely linked to the PSII light use efficiency and therefore the photochemical reflectance index is a remote indicator of the photosynthetic function (Gamon et al. 1997). With higher dissipating of excess light energy in IR64 at the low and medium RZT after 21 DAO compared to the high RZT these plants have a higher photosynthetic activity. In Chomrong there is significant differences between the values of PRI between all three root zone temperatures ranging from 18°C, 24.2°C to 28.4°C.

The figure 15 (32 DAO) shows a different picture compared to figure 14(21 DAO). In figure 15 at 32 DAO the medium RZT at 24.45°C of IR64 has the lowest values for PRI and according to the mechanistic understanding of the PRI the low PRI indicates lower light-use efficiency (Gamon et al. 1997). Not only in IR64 but also in Chomrong the same pattern of arrangement for the PRI values at the different RZT can be seen. The RZT has significant effect on the PRI values but there is no effect of the P-treatment.

Shimono et al. (2004) reported that during the vegetative growth the low RZT (~18°C) has no significant effect on the light-saturated leaf photosynthesis (P_{max}). The decrease in water content by low RZT can be linked to stomatal conductance (g_s) which lead to a decreased P_{max} although this influence of low RZT was higher in the reproductive growth period than in the vegetative growth period. According to Hasegawa et al. (1999), low RZT (16°C) decreases relative water content and transpiration. With a low RZT affecting the water uptake and hydraulic resistance higher evaporative demand in the reproductive period constrains the G_s and also the P_{max} more than in the vegetative period (Shimono et al. 2004). Similar results were found by Shishido and Kumakura (1994) in tomato grown under low RZT. The photosynthetic rate did not decrease under RZT in a range from 22°C, 15°C to 10°C but the amount of transpiration (g H_20/plant) decreased significantly. The reduction of dry matter production through low term low RZT in rice plants is the result of decreasing leaf area and not decreasing photosynthetic activity according to Nagasuga et al. (2011). These results were supported by findings by Shimono et al. (2002) that during the vegetative growth at low RZT limited leaf area and canopy radiation interception are responsible for the reduced dry matter increase in contrast to the radiation use efficiency which was not affected.

In this experiment the amount of P supplied to the plants had no effect on the PRI values in both varieties at the two measuring points (21 and 31 days after onset of RZT treatment). Makino et al. (1984) found that the photosynthetic rate of rice plants without P did not significantly differ from those with the full nutrient solution. In addition to the CO_2 exchange rate [µmol CO_2 min^{-1} leaf blade] the ribulose-1,5-biphosphate (RuBP) carboxylase content was measured because the primary step of the photosynthetic CO_2-assimilation is catalyzed by the RuBP carboxylase in rice plants. The results showed that phosphorus is not directly influencing the content of

RuBP carboxylase protein whereas nitrogen content is clearly correlated (Makino et al. 1984).

In experiments with sugar beets Abadia et al. (1987) found that low P-treatment reduced the leaf area by 80% and the total plant dry matter was reduced by 77% compared to the control treatment. Although the photosynthetic rate [µmol CO_2 $m^{-2}s^{-1}$] was decreased by 32% they state that the major effect of the low P-treatment on the accumulation of photosynthetic products was the reduction in photosynthetic surface expansion rate and not the decrease of photosynthesis per area. At the low P-treatment components associated with the PSI increased while components associated with the PSII have been reduced (Abadia et al. 1987). Xu et al. (2007) reported that under long-term P-deficiency the function of PSII was depressed and also the net photosynthesis rate (P_n) was heavily decreased. It is critical to compare these results to experiments where at least some P was contained in the low P nutrient solution whereas in the experiments like Xu et al. (2007) plants were under P-deficiency.

4.6. SPAD

In the first half of the experiment at 21 days after onset of temperature treatment (DAO) the SPAD values for IR64 were not significant different at the three root zone temperatures but at the 32 DAO measurement the SPAD values for the low RZT (19.7°C) were significant higher than the other RZT treatments. Similar results were found by Shimono et al. (2004) were in the beginning the SPAD values under low RZT were lower than the high RZT but later in the experiment it changed. Under low RZT (17.7 ± 1.7) the SPAD values were higher than in the high RZT (23.2 ± 1.7) which is similar to the low and medium RZT in this experiment.

The increase of the SPAD values under low RZT can be attributed to the change of SLA. According to Kuwagata et al. (2012) SLA is an indicator of leaf thickness and thicker leaves are connected to high photosynthesis due to high Chlorophyll content. To adjust for the different leaf thickness, the SPAD values can be adjusted like described in the Material and Methods. The adjusted values (SPAD*SLA) also show that in the low RZT (19.7°C) the values are significant higher for the variety IR64 and Chomrong. Increased SPAD values at the low RZT also decrease the negative effects on the light-saturated leaf photosynthesis (P_{max}). The decrease of stomatal conductance (g_s)negatively affects the Pmax but higher SPAD values have a positive effect on P_{max}. (Shimono et al. 2004).

Kuwagata et al. (2012) made the same conclusion that low RZT is the reason for high mesophyll photosynthetic capacity despite a decrease in stomatal conductance (g_s) and contributes to the maintenance of net assimilation rate (NAR). According to Shrestha et al. (2012) SPAD and PRI values increase with increasing N-supply to rice variety Chomrong. In this experiment, all plants received the same amount of N in the nutrient solution and therefore no effect of N on SPAD values is expected.

The statistical analyses show that the factor Pstat (amount of P in the nutrient solution has no effect on the SPAD values at every treatment and variety. When the plants showed no difference concerning the P-treatment than we also can conclude that the plants are sufficiently supplied with N. According to Samborski et al. (2009) canopy chlorophyll are strongly affected by N availability to the plant and often used to estimate plant N nutritional status.

The company Konica Minolta suggests a highly positive correlation between the leaf-blade N concentration and the measured SPAD values for their handheld SPAD 503 plus device used in this experiment. The change in SPAD values are the result of the low root zone temperature and this is clearly seen when the SPAD values are adjusted by the SLA values.

4.7. leaf area under varying VPD

As described in the experiment 1 the low RZT is suppressing the dry matter production by decreasing leaf area and the reduction of plant growth under low RZT is due to water stress (Kuwagata et al. 2012). According to Kuwagata et al. (2012) high evaporative demand under high VPD reduced the total dry weight and total leaf area but the effect was smaller than that of root zone temperature. In this experiment the leaf area was not significantly reduced at each of the two varieties.

In figure 19 the difference between the leaf area between of IR64 and Chomrong are visible. The variety IR64 has significant higher leaf area at the low and at the high VPD compared to Chomrong. The reason for the major difference in leaf area could be the natural growing conditions especially for Chomrong. In the VPD chamber with averaging air temperature of 31.9°C and root zone temperature of 30.17°C and 29.53°C for the high and low VPD treatment these conditions may not be suited for the variety Chomrong. According to Sthapit et al. (1995) Chomrong is grown at attitudes ranging from 1400-200 m above sea levels, is cold tolerant and adapted to high altitude rice systems, whereas IR64 is a lowland variety susceptible to drought (Ziska et al. 1996). The adaptation to the natural growing conditions and different conditions in this experiment could also be a reason for the low leaf area of Chomrong.

Providing additional P in the nutrient solution did not mitigate the stress by increased transpiration in the high VPD chamber of both varieties. With the factor Pstat having no influence on the leaf area and the values for different P-treatments in close proximity of each other the control P-treatment with 0.323mol P seems to be the most favorable P-nutrition.

4.8. Dry matter leaves, dry matter plant and specific leaf area

The negative effect of low RZT on the dry matter content of leaves as reported by Shimono et al. (2002) and Nagasuga et al. (2011) is not seen in this experiment because high RZT is used here. In this VPD experiment there is no significant difference of the dry matter weight of the different VPD levels. Same results are seen with the dry matter weight of the whole plant. Kuwagata et al. (2012) reports that there is a significant difference in the two-way ANOVA used in their experiments of total dry weight and leaf dry weight for plants grown under low and high humidity although the values at the high root zone temperature for low and high VPD level seem to be not significant.

In the experiments of Kuwagata et al. (2012) the NAR (g m^{-1} d^{-1}) was not significant different at 5% level between the high and low air humidity level of rice plant at 25°C root zone temperature. The NAR which is an indication for the dry matter production per unit leaf area according to Kuwagata et al. (2012) and any chance in NAR would result in change of dry matter production.

In figure 21 the dry matter of the whole plant at the high VPD level the low P-treatment has the highest value. At the variety Chomrong the effect of P is more pronounced than in IR64 where the values for the DM plant are increasing with decreasing amount of P in the nutrient solution. In contrast to the results by Seneweera et al. (1994) reported earlier in this experiment the low P-treatment of Chomrong at the high VPD has the highest plant dry matter. The only possible explanation is that the high DM of the plant for the low P-treatment is an effect of the high vapor pressure deficit. The plants were supplied always with sufficient water and the growth of the plants in this experiment should not be affected by drought. Although the water use efficiency (WUE, g TDW kg H_20^{-1}) of rice plants under different air humidity at 25°C RZT was significant lower at low air humidity (Kuwagata et al. 2012). Dry matter of leaves and plants of the two tested varieties are significant different with Chomrong having the lower dry matter in both leaves and total plant dry mass. Due to the growth conditions in high RZT and high air temperature in contrast to the original growing environment especially for Chomrong differences in dry matter is expected. In this experiment, also the roots were sampled and dry matter weight determined but no significant difference was found between the two VPD levels. There is a significant difference between the two varieties but also P had no effect on the root dry weight at the low and high VPD treatment.

In this experiment the specific leaf area is significant different between the low and high VPD level at both varieties. At the high VPD (low humidity) the SLA is low in both varieties and therefore this is an effect of the low air humidity. According to Kuwagata et al. (2012) SLA is an indicator of leaf thickness and thick leaves result in low SLA values. In this experiment the significant difference between the VPD level shows that low or high VPD has significant influence on the SLA values in both of the tested varieties. This indicates that not only the cold-tolerant upland rice variety Chomrong but also the lowland rice variety IR64 is affected.

In contrast to the reports of Kuwagata et al. (2012) where the humidity (VPD) has no effect on the SLA, in this study there is a significant effect of the high VPD treatment. The change in SLA could be the reaction of the plant to the reduced stomatal conductance and the severally increased daily transpiration. The stomatal conductance was reduced by dry air with low air humidity at the same RZT of 25°C and subjected to relative higher water stress than the low VPD treatment. In contrast to the humidity level at the same RZT the lower RZT at the high VPD level had the highest reduction of stomatal conductance (Kuwagata et al. 2012). Asch et al. (1995) reported that stomatal resistance (rs) is sensitive to relative humidity (RH) and that low RH treatments results in higher rs. This effect is also depending on the variety with stronger stomata response of some rice varieties. They also reported that low RH treatment increased the rs but did not affect the root/shoot water potential (SWP, -MPa). Kuwagata et al. (2012) reported that the daily transpiration (water uptake) per plant is 1.5- to 2-fold higher for the plants grown under high humidity irrespective of root zone temperature. In this experiment the leaf area and dry matter of the leaves there is no significant difference between the low and high VPD the SLA as a product of both is significant. Although there is no effect of the Pstat on the SLA the high P-treatment has the highest SLA values at the low VPD of both varieties and also the highest values in high VPD treatment of Chomrong.

4.9. P concentration in leaves under varying VPD

With increasing amount of P in the nutrient solution also the P concentration in the leaves increased. The hydroponic system used in this experiment allows the plants to take up the nutrients already dissolved in water in an optimal pH range (Sonneveld and Voogt, 2009). Rao and Terry (1995) described that when P is supplied to plants under very low P a rapid and massive uptake into the leaves can be observed. In this experiment the nutrient solution was changed weekly and therefore P was resupplied according to the P concentration in the nutrient solution. Different concentration inside the leaves are expected to be on a certain level due to the constant resupply and uptake of P. The high P plants received 100% more P than the low P plants and the reduction in P concentration from high to low P plants leaves was higher in the high VPD (-50% and -69% for Chomrong and IR64 respectively) than in low VPD (-28% and -41%).Zhou et al. (2009) used tomato plants for chilling experiments with different P contents in the nutrient solution. Based on the results from Stark et al. (2000) that sufficient P under optimal growing conditions might be not sufficient during chilling. The experiments of Zhou et al. (2009) used P-contents from 0 to 2.40 mM which is more than sufficient. Despite chilling stress the content of P [mg g^{-1} dm] did not reduce significantly at the sufficient P-treatment compared to the other lower P-treatments. In this experiment the plants of the high VPD treatment are under stress and the P-treatments of 0.323mol and 0.484mol P are able to maintain and increase their values for the P concentration at the high VPD level compared to the low VPD level. The results of this experiment are therefore in line with results reported by Zhou et al. (2009) and Stark et al. (2000) that under unfavorable conditions optimal or even luxurious P nutrition helps to maintain P concentration in the plant for optimal growth and development.

Shibli et al. (2001) did experiments with tissue culture to test the effect of salinity and phosphorus. An increased P did not only reduce the negative effects of salinity on plant growth and nutrient uptake but also improved the growth and nutrient uptake. Therefore, Shibli et al. (2001) described P as a key element to counteract salinity and therefore increased P supply to the plants can be considered as a management practice to reduce the negative impact of salinity.

In figure 24 the P content in leaves of IR64 and Chomrong are shown and the differences are very remarkable compared to the graph of the P concentration. Zhou et al. (2009) reported that the P content in the shoot, root, leaves and total plant dry matter [g $plant^{-1}$] is the highest in the chilled treatment with the highest P amount in the nutrient solution. Like in this experiment for IR64 plants under stress of high VPD the high P-treatment has the highest content of P in the leaves. The low P-treatment in the high VPD chamber is not sufficient to increase the P content under stress compared to the low VPD treatment. As with the concentration of P also the P content in the leaves is significant different between the two varieties at low and high VPD. The low P content in the leaves of Chomrong is the result of the low dry matter because the P concentration. This significant difference indicates that the environmental conditions might be unsuited for Chomrong or that the variety Chomrong is more efficient with the available P. Although the second suggestion is highly susceptible Chomrong is high yielding on low fertility soil compared to other rice varieties (Shrestha et al. 2011)

4.10. Number of tillers under varying VPD

The RH has no influence on the numbers of tillers in the low and high VPD treatment compared to the reduction of tillers described by Takamura et al. (1960) and Shimono et al. (2007). If there is an influence of RH than the tiller numbers at the low VPD treatment must be significant higher. In this experiment the root zone temperature is not changed artificially and through the small VPD chambers with additional lighting the RZT is high (~30°C). According to Ishii et al. (2011) the high RZT has not significantly increased the tiller production in rice.

Although Fageria et al. (1982) found that with increasing P-fertilization the number of tillers per m² increased in upland rice there is no evidence in this experiment that any increase in P concentration is increasing the number of tillers. There is a significant difference between the varieties in this experiment with Chomrong havinga significant lower number of tillers in both VPD treatments.

This result is in line with Chang and Vergara (1975) who reported that rice plants under upland conditions have fewer tillers and leaf area. Ishii et al. (2011) described the positive effect of high RZT during vegetative growth but also mentioned a lack of significant increase in tiller production. According to Yoshida (1981) the panicle number und spikelet density is determined by the tiller production and when there is a lack of tiller increase as described by Ishii et al. (2011) than the positive impact of high RZT on grain yield is reduced. Like in the first experiment higher temperatures of the root zones are prevailing in the VPD treatments.

4.11. Photochemical Reflectance Index under varying VPD

Lower PRI values at the high VPD indicates lower photosynthetic activity under water stress. The results of Ishihara and Saitoh(1987) state that the photosynthetic rate was much higher under lower VPD compared to the higher VPD treatment and therefore the main reason for reduced photosynthesis in midday conditions was the water stress related to the high VPD. In the measurements at 7 and 28 days after onset of the VPD treatment for both varieties VPD is significantly affecting the PRI values. The lower PRI values and therefore reduced photosynthetic activity under the high VPD treatment may be caused by the decreased stomatal aperture due to high evaporative demand (Ishihara and Saitoh 1987). Ishihara and Saitoh (1987) found a close relation between diffusive conductance and photosynthetic rate in rice leave following the same pattern. Under increasing light intensity, the photosynthesis was reduced in low humidity conditions.

Compared to the changing air humidity and light intensity in the experiments of Ishihara and Saitoh (1987) in this experiment the conditions inside the low and high

VPD chamber were not changed but the results are in line with Ishihara and Saitoh (1987).Hirasawa et al. (1988) showed that under increasing vapor pressure deficit the photosynthetic rate and diffusive conductance decreased and that the air humidity had a direct effect on both.

Similar experiments with rice leaves were made by Saitoh and Ishihara (1987). They found that with increasing VPD also the transpiration rate increased strongly but the photosynthetic rate, diffusive conductance and the intercellular CO_2 concentration was reduced. Same results were obtained by Ishihara and Saitoh (1987) when the photosynthetic rate under low and high VPD was compared and the photosynthetic rate was higher under low VPD. They concluded that the main factor for decreasing photosynthesis under midday conditions is water stress caused by high VPD.

The phosphorus concentration in the nutrient solution did not affected the PRI under low and high VPD. Although very low P leads to a reduction of stomatal conductance and therefore the photosynthesis should be negatively affected. Xu et al. (2007) found in rice that after 8 days of P starvation the reduction of stomatal conductance led to decreasing concentration of intercellular $C0_2$ and resulted in lower photosynthetic rate. In addition to the photosynthetic inhibition through stomatal limitation also disorder in the chloroplast photosystem could be the reason according the Starck et al. (2000). The analysis for tomato plants under different P treatment showed for all plants a positive highly significant correlation (*r*= 0.93) and as a conclusion they stated that the rate of photosynthesis is highly influenced by the stomatal conductance.

In the low VPD treatment where there is reduced water stress on the leaves and therefore also the stomatal conductance should be higher. Apart from the influence of P on the stomatal conductance Rao and Terry (1995) stated that the rate of photosynthesis is composed by the RuBP level and low P reduced the photosynthesis and the RuBP level. With resupply of P the photosynthesis and RuBP increased in the same pattern over time. In low-P plants more photosynthetic products are designated to non-P carbon compounds like starch than to sugar phosphates.

This adaption of P-deficient plants is involved in the maintenance of high rates of photosynthesis despite a reduction of P concentration in the leaves (Rao and Terry 1995). In contrast to Rao and Terry (1995) where the reduction was more severe than in this experiment this mechanism may help to maintain the photosynthesis of the low P plant on the same level like in the control. It is fair to say that the low P-treatment plants received enough P and 50% of the optimal P concentration did not lead to any negative effect on the photosynthesis. The photosynthetic performance of lower P concentration like 25% of the control would be interesting to analysis.

4.12. SPAD under varying VPD

According to Peng et al. (1996) the chlorophyll meter, often referred as SPAD, can be used to determine the leaf N status of rice and helps to manage the N fertilization to achieve high N efficiency at high yield levels. Shrestha et al. (2012) also found that with increasing N nutrition the SPAD and PRI values in rice increased. In this experiments all plants received the same N amount in the nutrient solution therefore no increase of SPAD and PRI due to N nutrition should develop. Previously mentioned in the PRI passage the high VPD treatment reduces the photosynthetic rate compared to low VPD (Ishihara and Saitoh 1987, Saitoh and Ishihara 1987). Kumagai et al. (2009) showed positive correlation of SPAD to chlorophyll content, rubisco content und maximum quantum yield of FSII (F_v/F_m). These positive correlations of photosynthetic parameters are possible indicators for the photosynthetic capacity of the rice flag leaves in the ripening stage according to Kumagai et al. (2009). Starck et al. (2000) reported that chlorophyll fluorescence is significantly reduced by chilling stress and imply a reduction of the PSII photochemical reaction efficiency.

The adjustment of the SPAD values described by Peng et al. (1993) shows that the significantly higher SPAD values found in high VPD treatment at the 14 DAO measurement (Figure 29) are due to thicker leaves. When the SPAD values are adjusted with SLA values there is no significant difference between VPD treatments. The SPAD values are calculated based on the intensity of transmitted light but leaf thickness is not taken into consideration (Peng et al. 1993). At the end of the experiment the SPAD values at the 28 DAO measurements show no significant difference between VPD treatments and when these SPAD values are adjusted to the leave thickness the high VPD treatments have significant lower values than the low VPD. The significant lower SPAD*SLA values at the 28 DAO implies the question if any adjustment to the leave thickness with the SLA values is appropriate. Peng et al. (1993) used the specific leaf weight (dry matter [g] * leaf area [cm^{-2}]) for the adjustment but the result is the same as with SLA [cm^2/g].

It is questionable if SPAD values from the measurements in the beginning of the experiment are suited to multiply with the SLA values which are estimated at the end of the experiment. It is possible that the reduced leaf area and higher dry weight under high VPD is developing over time as an adaptation to the low relative humidity.

5.Hypothesis

1. Lower root temperature inhibits the growth, the uptake of phosphorus and has an effect on the photosynthesis because phosphorus is directly involvedin the regulation of the Calvin cycle and triose phosphate export from the chloroplast. With an increasing amount of phosphorus in the nutrient solution the negative effect of low root temperature might be mitigated.

The growth of the plants is measured in leaf area and dry weight of leaves. Low RZT significantly reduced leaf area in IR64 and Chomrong compared to the higher root zone temperature. The increase in leaf area with increasing root zone temperature occurs in a linear fashion. High P nutrition has positive influence on leaf area in Chomrong especially at the low root zone temperature. Although there are no significant effects of P in IR64 the results of Chomrong suggests that at low RZT additional P is beneficial for the leaf area and increased canopy size might influence carbohydrate supply to young leaves, leaf appearance and expansion. The dry matter of the leaves is increasing in a linear fashion with increasing RZT in IR64 and for the control (Pstat opt) treatment in Chomrong. Low P-treatment reduced the dry matter in IR64 in every temperature treatment and in Chomrong in the low and high RZT. In contrast to the low P-treatment additional P in the high P-treatment increased the dry matter of the leaves in the low and high RZT in Chomrong. The strong increase in dry matter in the high P-treatment under low RZT is only present in the variety Chomrong. At the low RZTP concentration in the leaves is significant higher than the higher RZT in both varieties. The increase in P inside the nutrient solution leads to significant increase of P concentration inside the leaves. At the low RZT Chomrong showed an improved uptake at low P due to adaptation to lower RZT resulting in the same P concentration as the control. The reduced leaf area and dry matter content of the leaves are the main reason for the reduced P content in the low RZT compared to the higher RZT treatment which have higher leaf area and dry matter. In the PRI measurements, no significant effect of low root zone temperature in all measurements can be seen. Although the photochemical reflectance index is higher in the measurement 21 days after onset of treatment, indicating higher xanthophyll cycle activity and higher stress. PRI can be used as a remote indicator of photosynthetic function and with the mechanistic understanding that low PRI results in lower light-use efficiency. In later measurements at 32 DAO the low and high RZT are significantly higher than the medium RZT. In multiple sources (Shimono et al. 2004, Shishido and Kumakura, 1994, Shimono et al. 2002) stated that photosynthetic rate is not decreased under low root zone temperature but that low RZT limit leaf area and canopy radiation interception which lead to reduced dry matter increase.

The SPAD measurements at the low RZT where lower or similar compared to the medium and high RZT but at the end of the experiment (32 DAO) IR64 showed higher SPAD values at the low RZT than the higher RZT treatments. When the SPAD values are adjusted with the SLA values accounting for the leaf thickness than the SPAD*SLA values at the low RZT are significant higher than the high RZT (IR64) and medium/high RZT (Chomrong). The amount of P in the nutrient solution had no effect in any treatment or variety.

2. Tillering is highly impaired by nitrogen or phosphorus deficiency and the nutrient status of the plant should be related to the number of tillers. In low P-treatments a lower number of tillers should be produced compared to the optimum and high P-treatments.

In both tested varieties, the root zone temperature is significantly affecting the number of tillers and the lowest number of tillers are counted in the low RZT treatment. Like described by Ishii et al. (2011) the high RZT does not have a significant effect on the tiller number. Although there is no significant difference between the P-treatments in both varieties the effect of different P-treatments is visible in Chomrong where the high P-treatments have the highest number of tillers in every RZT. The tillering behavior of IR64 can be described as temperature depended while Chomrong is also P-depended.

Different VPD treatments with low and high vapor pressure deficit has no effect on the number of tillers. Increasing the concentration of P in the nutrient solution did not increased the number of tillers in this experiment. Differences in tillering rate between the two cultivars with Chomrong having significant lower number of tiller based on the genetics of upland cultivars.

3. Under high vapor pressure deficit higher content of phosphorus in the nutrient solution and better availability to the plant helps to control the loss of water through transpiration. The low relative humidity under high VPD will influence the photosynthetic activity, P concentration in the leaves and dry matter through water stress.

In this experiment the applied measuring devices are not able to measure the loss of water. The amount nutrient solution left in the pot before the weekly change of nutrient solution was not measured and the leaf area was determined destructively in the end of the experiment. During the experiment the leaf area or plant dry matter were not determined due to the high number of plants which would be necessary to have sufficient repetitions.

Significant differences of the leaf area, leave dry matter and plant dry matter between the lowland rice variety IR64 and upland variety Chomrong are visible under high air and RZT. These differences are due to adaptation to different altitudes and climates. Different VPD and P treatments have no significant effect on the leaf area and leave dry matter in both tested varieties.

The plant dry matter is not affected by the VPD or P level but when the high VPD treatment of Chomrong is analyzed there is a significant difference between the low and high P treatment. Higher plant dry matter of Chomrong under high VPD could be an effect of the high VPD and continuous uptake of water and nutrients. Differences between the varieties are explained by their adaptation to different climates.

Low specific leaf area indicates thick leaves and under high VPD (low air humidity) low SLA values are present. In this experiment the influence of VPD on the SLA is clearly shown because in both varieties low SLA values are determined. Although there is no significant effect of P in Chomrong the highest SLA values in both VPD treatments are with the high P treatments.

The P concentration in the leaves in IR64 and Chomrong is only influenced by the amount of P in the nutrient solution. The water stress conditions due to high VPD treatment the optimal and high P treatment are necessary to maintain or even increase the P concentration in the leaves to maintain plant growth and development. As described in the literature plant under stress like chilling (Zhou et al. 2009) and salinity (Shibli et al. 2001) improved P nutrition is necessary under unfavorable conditions. The reduction in P content in leaves is due the low dry matter content.

The lower PRI values at the high VPD levels indicate lower photosynthetic activity due to water stress. In both measurements at the beginning and in the end of the experiment the VPD is affecting the PRI significantly. The photochemical reflectance index served as a remote indicator for photosynthesis and accordingly there must be also a reduction in photosynthesis. These results are in line with many researchers (Hirasawa et al. 1988, Saitoh and Ishihara 1987, Ishihara and Saitoh 1987) who proved that under increasing vapor pressure deficit the photosynthetic rate is decreasing. Providing additional P in the high P-treatment did not affect the PRI but also low P-treatment had no effect. Very low P or plants under P-deficiency is affecting the stomatal conductance (Xu et al. 2007) and therefore affecting the photosynthesis. In addition to the reduction in stomatal conductance Rao and Terry (1995) reported that low P reduces the RuBP and photosynthesis. Compared to the results found by other researchers in this experiment the low P treatment with 0.161mol P is clearly higher therefore comparing results are made simply.

The increase of SPAD values during the experiment indicates a change in leaf thickness which is expected for the plants in the high VPD treatment chamber. When the SPAD values are adjusted then there is no significant difference and even significant lower SPAD*SLA values are found for the last measurement. In this experiment, every treatment received the same amount of N in the nutrient solution and therefore no difference in chlorophyll content within the VPD treatment is measured.

6. Conclusion

The positive influence of high P-treatment at low root zone temperature of Chomrong indicates positive response of this variety to improved P availability. Higher leaf area which might lead to improved quantity of photosynthetic produce and higher number of tillers might result in higher yield. Positive effects on the yield and also a statistical analysis to show which factor has significant effect can be determined in a longer experiment. The effect of even lower root zone temperatures of 15°C and lower P-concentrations in the nutrient solution would be interesting to determine. In a small separate experiment with root zone temperatures close to 15°C rice plants showed anthocyanin colorations at the base of the stem under low-P nutrition.

Field trails under low RZT are needed to prove the effect of increased P-fertilization on the plant growth because in a hydroponic system perfect condition are created. P-fixing soils found in the Subtropics and Tropics are a special challenge when it comes to P fertilization. Therefore, precise placement of fertilizer containing P can be an option for increased P availability. In industrialized countries farmers, can buy and maintain seeding equipment which is capable of seeding and placing fertilizer at the same time. When we think about the upland farmers of Nepal these people might not have the financial possibilities and access to such machines. Low tech and low cost fertilizer placement machines might be a part of the solution to increase the P availability to the plants on P-deficient or P-fixing soils. Apart from the technical solution P-efficient cultivars which are adapted to the local climate (e.g. cold tolerance) should be selected. Apart from all the aspects of climate and tolerance the grain color and taste must be approved by the people if the variety are to be successfully introduced into rotation.

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