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Growth responses of irrigated rice to root-zone chilling under different Vapor Pressure Deficit (VPD) conditions.

Master Thesis

by

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Abstract

Rice is staple food for more than half of the world, which can be grown from cool-temperate to dry regions. Production of rice is limited by different abiotic stresses, temperature is considered one of the major factors that reduce growth and development at high and low temperature extremes. Consequently knowing the plant responses under different climatic conditions might lead to the development of resistant breeds for these environmental conditions. This experiment was designed to assess the effect of low root temperature (LRT) and vapour pressure deficit (VPD) on leaf appearance rate, morphological responses and growth rate. Three different temperatures (17 ^{0}C, 23.2 ^{0}C and 27.7 ^{0}C) at root zone and two different VPD level (high and low) at shoot zone were applied in two rice varieties of different origin. Leaf appearance rate (LAR) as well as other observations like plant parameters, absolute growth rate (AGR), leaf area expansion rate (LER), leaf mass fraction(LMF) and stem mass fraction(SMF)were decreased under low root temperature(LRT), which remained increased under medium and high temperature. LAR was not markedly influenced by VPD level under all temperature treatments. Varietal differences were observed in many cases. Under LRT, LAR was delayed more in Chomrong than in IR64. Chomrong responded well under low VPD whereas IR64 under high VPD. Integrated effects of LRT and VPD were also noticed. In several cases plants responded well under LRT and high VPD conditions, which was relatively more in IR64 than in Chomrong.

Keywords: **Irrigated rice**, **LRT**, **meristem**, **VPD**, **LAR**

Erklärung

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Hohenheim, den 29. August 2014

Signature ___________________________

Bhav Narayan Jha

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1. Introduction

Rice (*Oryza sativa L.*) is a staple food for more than 50% world's population. It is probably world's most diverse and versatile crop. It can be cultivated in different areas from tropical to cool-temperate regions. It can also be cultivated in dry regions such as California (USA), New South Wales (Australia), Punjab (India) and Egypt (Kuwagata et al., 2012). Rice the second most cultivated cereals globally after wheat. It is estimated that by 2025 A.D rice production will increase by sixty percentages as compared to the current production level to meet the demand of the population (Fageria, 2007). Different environmental stresses resulting from global climate change can influence crop production system all over the world. To fulfill the demand of growing population it is necessary to think about all possible solutions that can overcome from different environmental condition. Determination of growth responses of crops under different climatic conditions might lead to develop resistant breeds for the specific environment.

Rice is sensitive to water shortage (Bouman and Tuong, 2001), so it is grown mostly under flooded conditions, consequently can suffer from cool water stress at any time during growth processes (Shimono et al., 2002). The root zone temperature of the paddy field remains cool even though rapid rise in air temperature due to large heat capacity of the water and soil. Rice plants suffer from chilling stresses attributed by reduced water uptake (Kuwagata et al., 2012), however it can be acclimated after 5days of continuous low root temperature by acquiring a water uptake mechanism (Ahamed et al., 2012), which shows the growing point (that remain inside the water) other than the root is responsible for chilling stress in rice.

Chilling stress is not only major constraint in rice production in cool climates but also in dry tropics and subtropics due to different irrigation management practices. In those areas more rice need to be grown with less water because competition for water between agriculture and industry is high, therefore water-saving irrigation have been tested and found reduction in plant growth caused by low root and meristem temperature (Stuerz, 2014).

Temperature effect on growth and yield are complicated because on one hand rise in temperature up to 30°C stimulates the growth (Cutler et al., 1980), but crop duration is shortened (Dingkuhn et al., 1995); On the other hand via accelerated development leading less tillers per hill are produced (Shrestha et al., 2012). Extreme temperatures lead to yield reductions due to heat (>35°C) or cold (<18°C) induced sterility, one of the major constraints for rice production in the dry tropics and subtropics (Stuerz, 2014).

Plant growth requires coordinated co-operation between shoot and root. Air Humidity in shoot zone determines the effect of water, salt and temperature stresses (Capell and Dörffling, 1989; Lauter and Munns, 1987; Mizrahi et al., 1971). Arid conditions enhance such stresses leading crops less salt tolerant under dry than under humid conditions(Fageria, 1985). On the other hand, low air humidity up-regulates expression of aquaporins in both roots and shoots (Kuwagata et al., 2012), which amplify acclimation process under long run low root temperature treatment in rice (Ahamed et al., 2012).

2. Objectives

This experiment was conducted based on the research done by Stuerz (2014) on irrigated rice in the Sahel. According to their hypothesis, water saving irrigation decreases the soil and meristem temperature and led to changes in canopy microclimate. Considering the difficulties to measure relative air humidity inside the canopy in the cropped field, this experiment was conducted with the specific objectives:

- to investigate leaf appearance rate (LAR),
- to analyze morphological responses, and
- to analyze growth rates under low root temperature and different VPD conditions in two rice varieties with different origin .

3. Literature review

3.1 Influence of root zone temperature on rice production

3.1.1. In general

Low root zone temperature is one of the main constraints in rice production in cool climates. Continuous water supply and excessive water drainage to and from paddy fields decrease the flooded water temperature even on sunny days (Nagasuga et al., 2011). Air and water temperature influence the Growth and development of rice crop (Dingkuhn et al., 1995), particularly for irrigated rice thermal environment of the growing point should be considered for crop ontogeny studies because shoot meristem is exposed to water temperature until booting stage afterwards internode elongation lifts the apex above water surface. There are several reports that address rice growth at suboptimal air and flood- water temperatures. Most of the results are based on either insufficient supply of water from the root to the shoot or temperature effect on growing point. Despite these findings, the underlying mechanisms are not yet fully understood.

3.1.2. Phyllochron and leaf appearance rate

Phyllochron is defined as the interval between the appearances of two successive leaves during the development of rice which change under different growth condition (Itoh and Sano, 2006). The three processes comprising the phyllochron are: cell division that form the leaf primordium, cell division of the intercalary meristem of the expending leaf primordium and gowth of cells derived from the intercalary meristem resulting in the leaf lamina and sheath; first two process are predominantly controlled by temperature and cell expansion is much more dependent on carbohydrates, water, nutrients and light (McMaster et al., 2003). Phyllochron depends on the sufficiency of current photosynthesis (source) to meet the demands of the plant for growth (sink) (Birch et al., 1998). The phyllochron and plastochron (interval of leaf initiation) are not always same duration in the family Poaceae. Usually, their phyllochron is longer than plastochron and phyllochron is synchronized with the plastochrone, which is a main feature of rice shoot development during vegetative stage (Nemoto et al., 1995).

Leaf appearance is one of the major development parameters, which can be used to determine temperature response (Ritchie and NeSmith, 1991). Although air temperature affects leaf temperatures whereas water temperature affects the temperature of the shoot base and rooting zone which exists below the water (Shimono et al., 2007). Despite many findings regarding LRT attributed water uptake inhibition (e.g. Kuwagata et al., 2004; Murai-Hatano et al., 2008), temperature at growing points have been rarely considered. Yin et al. (1996) reported that meristem temperature control the LAR. Ellis et al. (1993) found delayed LAR at 20 ^{0}C compared to 24^{0}C and 28^{0}C temperature.

The physiologically meaningful age of a rice plant can be determined via counting the leaves of the main culm. The leaf blade is absent in the first leaf, but from the second leaf, all leaves develop a leaf blade. From the third leaf, a new leaf emerges after the preceding leaf has fully developed (Yoshida, 1981). The appearance of consecutive leaves can be described in two ways: either when the leaf tip exceeds the leaf sheath or with the appearance of the ligule when the leaf is considered fully developed.

Most varieties develop about 10 to 18 leaves on the main tiller. Phyllochron of tillers closely resembles the phyllochron of main tiller (Jaffuell and Dauzat, 2005). Under most of conditions, the number of leaves is constant in photoperiod-insensitive varieties. Rice is composed of leaves which are physiologically different in age and activity at a given time. A leaf emerges every 4 to 5 days before the initiation of panicle primordial, afterwards it emerges every 7 to 8 days. The life span of elongated leaves varies with position of the leaves. The life span of upper leaves is longer than the one of lower leaves (Yoshida, 1981). The maximum value of phyllochron can be found around the time of floral initiation (Itoh and Sano, 2006), which is associated primarily with inflorescence initiation (Katayama, 1951).

The effect of root temperature on phyllochron has been rarely studied. Itoh and Sano (2006) found that, the phyllochron was longer under low temperature than under high temperature conditions, indicating that the time necessary to form each phytomer was longer in low temperature than in high temperature conditions for all tested rice genotypes. Crop duration determine the agro-ecological and agronomic fit of rice cultivars which can be influenced by photoperiod and temperature, low water temperature leads to delayed germination and the appearance of the first two to five leaves (Sié et al., 1998). It has been shown that effects on rice crop duration have a proportional effect on leaf number, whereas temperature mainly affects duration (Yoshida, 1981). The underlying theory is that low temperatures increase crop duration mainly through delayed leaf appearance (Sié et al., 1998). Thus it will be worth to see the effect of root or meristem temperature on leaf appearance of rice under controlled conditions.

3.1.3. Growth

Water temperature is a major limiting factor for growth and yield of rice grown under cool climates, particularly during vegetative growth, slowed the emergence of new leaves and of tillering, and decreased leaf expansion leading more thick leaves, resulting in decreased canopy interception of radiation which may alleviate reductions in photosynthetic rate and decreased dry matter production (Shimono et al., 2002). Baker and Allen (1993) showed that rice canopy gas exchange is affected by warm water, but did not mention cool water effect. Nishiyama (1978) mentioned that water temperature has a larger influence on leaf growth than air temperature.

Rice plant grown under LRT reduces the ability of water uptake by root to shoot, leading low leaf water potential and thus, decreased transpiration rate and stomatal conductance (Kuwagata et al., 2004). Transpiration is the loss of water in the form of vapor from plant surfaces which directly related to amount of growth and transpiration varies with climate, soil moisture, variety, growth stage and growth duration of the plant (Yoshida, 1981). Tang and Boyer (2002) mentioned that leaf growth was regulated by water potential gradient between xylem and expending cells in growing parts, which is very sensitive to water supply from roots.

Nagasuga et al. (2011) found that low temperature treatment of root significantly decreased the root dry weight, root length, total root surface area and specific area of roots, indicating that water uptake from root is controlled by both the water permeability of the root surface and the root volume. However there are few studies on the relationship between LRT and root volume in rice. On the other hand, Murai-Hatano et al. (2008) showed that root hydraulic conductivity in rice (Lp_r), which relates to its permeability, decreased with decreasing root temperature and that (Lp_r) decreased dramatically at temperature below 15^0C and this is linked to the function of aquaporins. Aquaporins are transmembrane proteins involved in regulating the inter- and intracellular water flow in various microorganisms, animals and plants (King et al., 2004).

Arai-Sanoh et al. (2010) observed root shoot growth at four different soil temperature (18^0C, 25^0C, 32^0C and 37^0C) and found that dry weight of shoot and plant height decreased and the number of tiller increased in 37^0C at 21 days after treatment in the stage from late tillering to panicle initiation. In addition, they also found high abscisic acid (ABA) production in roots under high soil temperature leading reduction in diffusive conductance. The ABA level has been shown to be increased by water stress, and inhibit transpiration through stomatal closure (Davies and Zhang, 1991). However, it is still unclear whether different growth limiting mechanisms caused by the integrated effects of low temperature on the root zone and shoot meristem near the soil surface or the effect on the root system (root temperature) alone, because in many studies flooded water was cooled in the paddy field accompanied with a decrease in air temperature (Nagasuga et al., 2011). Under field condition, Stuerz, (2014) found that lower minimum temperature of the soil and at meristem level caused reduction of gas exchange and thus assimilation rate, also reduction of leaf area expansion rate and thus leaf area.

3.1.4. Allocation

Plants consist of leaves that fix carbon (C), stems that give mechanical support and provide a hydraulic pathway and roots that take up nutrients and water and anchor the plant, but the proportion of the biomass present in the various organs, which is described as biomass allocation, may vary over time, environmental conditions and species (Poorter et al., 2011). There are different expressions that are used to specify the biomass allocation in plants like the shoot: root or root: shoot ratio which is frequently used. Plants allocate more biomass to roots, if the required growth factor is extracted from the root (e.g. nutrients, water) and more to shoots, if the required growth factor is from the shoot (e.g. light, CO_2) and this functional aspect might be due to increase in plant growth by acquiring most limiting factors (Poorter et al., 2011). Results from (Kuwagata et al. 2012) showed that dry matter allocation to the root under LRT was significantly reduced, but allocation to the leaf blade was not affected. On the other hand Nagasuga et al. (2011) found significantly decreased leaf dry weight and dry matter allocation to the leaves under LRT.

3.2 Influence of VPD on rice production

3.2.1. In general

Humidity of the air can be defined in different ways, like vapour pressure, water content, vapour pressure deficit or relative humidity (Penman, 1995). Here we characterize humidity as vapour pressure deficit (VPD). VPD is the difference between saturation vapor pressure at a given temperature and the actual water vapor pressure and it drives water movements from inside the leaf to the atmosphere. The epidermis of leaves is covered by an outer layer, the cuticle, which controls both water and CO_2 diffusion. Stomata are the minute openings at the leaf surface that balance water loss and carbon gain (Buckley, 2005). At air levels of carbon dioxide concentration, increasing VPD reduces leaf stomatal conductance in most species, which limits transpiration rates and affects the energy balance of the vegetation (Bunce, 2006). Stomatal behavior under different VPD levels can be explained with two different mechanisms. The feedforward hypothesis assumes that stomatal conductance declines as VPD rises, which helps to minimize transpirational water loss by plants (Farquhar, 1978), and feedback response of stomata which assumes that as VPD increases, stomatal conductance decreases due to a direct increase in transpiration (Mott and Parkhurst, 1991; Moteith, 1995). Both mechanisms are based on the fact that high VPD decreases stomatal conductance caused by stomatal closure mediated by high transpiration demand under high VPD and thus a reduction in water potential somewhere in the plant (Bunce, 2006).

3.2.2. Phyllochron and leaf appearance rate

The physiological age is very much affected by environment. Even for the same location and variety, variable weather conditions affect the rate of seedling growth (Yoshida, 1981). So far, not much study exists explaining the effect of humidity on the phyllochron. The phyllochron decreases with temperatures, light intensity, and nutrition whereas it increases with planting density but humidity has different effects on the phyllochron, depending on the temperature regime (Nemoto et al., 1995), however it is not clearly mentioned the underlying mechanism how humidity and temperature integrated to change phyllochron of rice. Ford and Throne (1974) and Schüssler (1992) found that an increase of relative humidity causes an increase in height and leaf area of many plants.

Hirai et al. (2000) conducted experiment in rice plant age of 3 leaves for 10 days in a 12-h light/12-h dark cycle, and exposed to 60% (low) or 90%(high) relative humidity during the light and dark period in all combinations and found significantly increased rate of leaf emergence, leaf area, leaf blade length, plant height, total root length, the number of roots and dry matter production under high humidity in light and darkness compare to low humidity in both light and dark periods.

3.2.3. Growth and allocation

The role of VPD on growth and development of plants is difficult to analyze. Dry air mediated water deficits in plants restrict growth (Kozlowski, 1964). On the other hand saturated air may have negative effects, favoring hormonal imbalance (Pareek et al., 1968) which might lead to reduced plant growth compared to lower humidity.

3.3 Integrated effects of LRT and VPD on rice production

3.3.1. In general

Plant exists in a soil-plant-atmosphere-continuum, so metabolic process is not only regulated by available shoot environment, but also regulated by root environment. In field condition, Stuerz (2014) found lower minimum canopy relative humidity, which is associated with decreased transpiration rate due to lower minimum soil temperature and meristem temperature under water saving irrigation technique. Cooper (1973) showed that soil temperature can influence photosynthesis and respiration and yoshida and Shioya (1976) mentioned that the growth and activity of roots are closely related to the aerial parts. Several research have been conducted describing effects of single factors such as low root temperature (e.g. Nagasuga et al., 2011), relative humidity (e.g. Asch et al., 1995), VPD (e.g. Ohsumi et al., 2008), or radiation (e.g. Campbell et al., 2001), on stomatal conductance ($g_{s)}$ and assimilation (A), but the way plants integrate all these different climatic factors is complex (Shrestha et al., 2012) and to date still poorly addressed. Wide level of understanding on this integrative process will help determining the growth limiting factors in a specific environment which can help improvement of crop management and to achieve breeding target (Stuerz, 2014).

Photosynthetic rate in plant is estimated by the amount of CO_2 supplied via stomata and the capacity for CO_2 fixation into carbohydrate (Shimono et al., 2004). In addition, they also found that low water temperature decreased plant water content and bleeding rate which indicates that the water balance between water uptake from roots and water loss through transpiration was influenced by low water treatment. Chhun et al. (2007) found that the saturated humidity surrounding the shoot of rice seedlings promote root branching by impacting phloem-based auxin transport from shoot to the root and this provides basic mechanisms for how environmental signals could affect root architecture by triggering auxin transport. Azuma et al. (1991) observed that shoot elongation was enhanced under saturated humidity conditions in floating rice.

3.3.2. Growth and allocation

In many case, the reduction in shoot growth mediated by the inability of the root system to translocate the resources adequately (Karmer and Boyer, 1995). E.g. by adjusting the nitrogen supply to the roots can help to control shoot nitrogen and plant growth (Dood, 2005). Kuwagata et al. (2012) found up regulation of aquaporins in the leaves and the roots under LRT and low humidity conditions. Recently, Ahamed et al. (2012) found acclimation to LRT by acquiring a water uptake mechanism which is associated with up regulation of aquaporins. On the other hand, Murai-Hatano et al. (2008) reported that activities of aquaporins are more responsible than their abundance for reduced root hydraulic conductivity under LRT. However, the underlying mechanism of VPD and root temperature interaction (i.e. shoot to root signaling under LRT conditions) is still unclear.

Not a lot of research exists that addresses growth and biomass allocation of rice under varying environmental conditions considering more than one parameter. Kuwagata et al. (2012) observed the effects of low (13^0C) or high (25^0C) water temperature treatments under humid and dry condition on physiological and morphological parameters in the controlled environment of a growth chamber, and found that LRT exposure reduced dry matter production and leaf area expansion and effects were larger at low humidity than at high humidity and concluded that rice plants acclimate to low air humidity mainly by using physiological processes (such as stomatal conductance and aquaporin expression) whereas LRT leads to changes at both the physiological and morphological levels (such as dry matter distribution to each organ and specific leaf area).

4. Material and Methods

The experiment was conducted from 10th May to 24th July 2013 in the greenhouse of the University of Hohenheim. The plants were grown for 8 weeks in the greenhouse using a hydroponic system.

4.1 Plant material used

Two rice varieties (Chomrong and IR64) originating from regions with different climatic conditions were provided by the institute for crop production and agroecology in the Tropics and Subtropics - Crop water stress management at the University of Hohenheim. Details about these two varieties are shown below in Table1.

Table 1: Selected varieties for experiment, sat=sativa, jap=japonica, ind=indica. Source: Schlegel (2009) (adapted).

Varieties	Species/ subspecies	Type	Duration	Country origin	Special properties
Chomrong	sat/jap	Traditional	Short	Nepal	Cold tolerant
IR64	sat/ind	Improved	Short	Phillipines	Intern.check

4.2 Plant Growth

The seed were laid on filter paper with de-ionized water in two small boxes. The boxes were kept in the greenhouse under HPS Sodium high pressure lamp (Phillip SON-T 400 WAT) for 6 days until seeds were germinated. Seedlings were then planted into multi-pot-pallets filled with sieved sand. The plants were watered until the sand was saturated at the first day. From the second day, plants were irrigated with 25% nutrient solution (Table 2). Seedlings were kept under 12 hour photoperiod conditions. 11 days after germination, plants were transferred into the hydroponic system. The root of each plant was carefully washed before the transfer to prevent contamination of the system. Almost all emerged plants were at 3-4 leaf stage. Only well-established plants were selected for further investigation.

4.3 Nutrient Solution

The nutrient composition used in the experiment was prepared according to Yoshida et al., (1976).

Table 2: Nutrient Solution. Source: Yoshida et al. (1976) (adapted).

Element	Chemical	Stock (g)/L	Stock / final (mL/L)	Solubility(g/L)
N	NH_4NO_3	114.29	1	2089
P	$NaH_2PO_4.2H_2O$	50.37	1	850
K	K_2SO_4	89.14	1	111
Ca	$CaCl_2$	146.73	1	986
Mg	$MgSO_4.7H_2O$	405.64	1	710
Fe	FeNa-EDTA	15.08	1	N.N.
Mn	$MnCl_2.4H_2O$	1.88		700
Mo	$(NH_4)_6.\ MO_7O_{24}.4H_2O$	0.09		430
B	H_3BO_3	1.16	1	50
Zn	$ZnSO_4.7H_2O$	0.04		965
Cu	$CuSO_4.5H_2O$	0.03		203

The nutrient stock solutions were prepared manually in the institute's lab. All the micronutrients were dissolved in 0.5ml HCl (0.5M). 1L of stock solution was made by adding distilled water. 1ml for 1liter final solution was taken from stock solution. All the macronutrients were made separately (1L for each macronutrient) adding distilled water to prevent precipitation during preparation of the nutrient solution. 1ml was taken from each stock solution for 1L final solution. The strength of the solution was adjusted according to the demand of the plant. The nutrient solutions were frequently replaced with increasing strength from 25 % (first week), 50% (second week) and 100% (rest of the week). The pH of the nutrient solution was adjusted to 5.0 ± 0.2 with either NaOH 0.1M or HCl 1N.

4.4 Experiment Set-Up and Design

Prior to the experiment, technical set-up was installed using PVC pipes, boxes, polypipes for water circulation and water chiller cooling systems (SeaChill TR10 1/8 HP Aquarium chiller). PVC pipes of 7 cm diameter were cut in 23 cm long tubes. 15 pipes were put together and fixed by melting the contact-side borders with a soldering iron. The pipe trays formed were introduced in rectangular boxes with 40 cm width 30 cm length and 30 cm height. 12 such boxes were prepared (six boxes for each chamber) .In the uppper part of each 23cm long pipe, half-split foam (7 cm in diameter, 3cm high supplied by OBI) was inserted in order to fix each plant individually. The seed and roots were protruded from the foam, allowing the root and meristem to be in contact with nutrient solution.

The experiment was designed containing

- two varieties
- three root zone temperature levels
- two VPD levels

Since plants were sampled in five samplings with three replications each, the total setup contained 180 rice plants. Six rectangular boxes were randomized within the chamber (two boxes for each treatment) as shown in figure 1. 15 plants from each variety were randomly allocated in two boxes by 8+7 order. If one box is with 8 chomrong plants and 7 IR64 plants then another was reversed. One plant from each variety was labeled in each box for phyllochron observation.

4.5 Treatment

4.5.1 Temperature

Nutrient solution of three different levels of temperature (figure 5) was conducted through a circulation system. Inlets and outlets were arranged in each box for continuous circulation. Since nutrients were taken up by the plants from the solution, therefore nutrient solution was replaced every week during the experimental period. The circulation of nutrient Solution in each system is shown in figure 1.

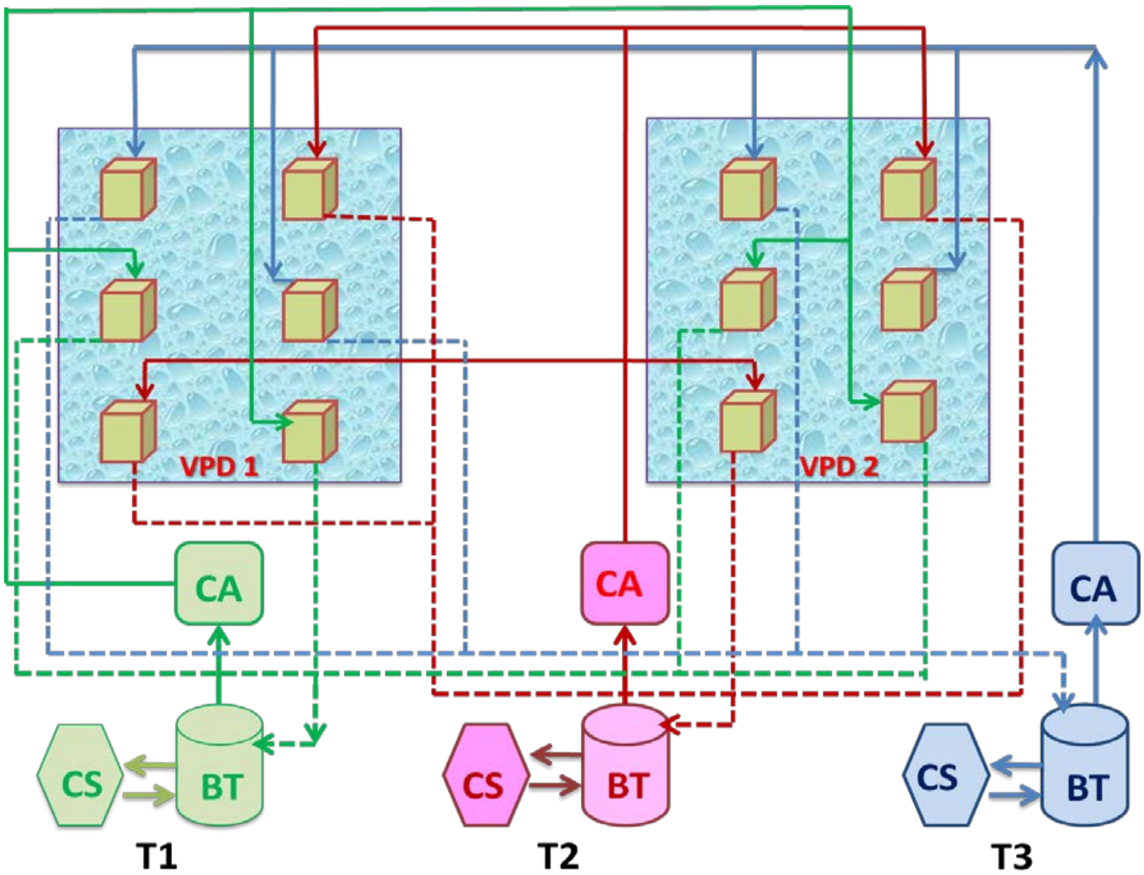

Figure 1: Experiment set- up and nutrient circulation system (Green- low root temperature, Red- medium root temperature, Blue- high root temperature; solid lines- active pathway and dotted line- passive pathway; BT-buffer tank, CA-control apparatus, CS-cooling system).

- **Active Pathway:** Buffer tank (BT) of 60L was fitted in each system. Nutrient solution from buffer tank was pumped by pump (variolux pumpe, 350 W) through polypies in four boxes (two boxes in each chamber) represented by solid lines in above figure. Flow control apparatus (CA) were fitted to supply same amount of nutrient. Four inlets were connected with this apparatus and passed to the top of the boxes.

- **Passive Pathway:** Outlet of each box was ended to the buffer tank represented by dotted lines in above figure. As soon as nutrient solution reached at the top level of box, it flows passively through polypipes into the buffer tank. Solution from buffer tanks pumped into the water chiller cooling system (CS) where get cooled according to adjusted temperature and then returned back to the tank. Three different such nutrient circulation system conducted for each temperature treatment (figure 1).

4.5.2 VPD

Two levels of VPD were established in separate plastic covered chambers (80*80*100 cm). HPS Sodium high pressure lamps (Phillip SON-T 400 WAT) were adjusted on the top of each chamber.

- **<u>Low VPD</u>**

  This VPD chamber was closed and supplied with fog. Fog was generated outside the chamber with a nebulizer and passed through PVC pipes into the chamber as shown in figure 2. With the help of a humidity sensor, relative humidity was automatically adjusted to 80% relative air humidity in the chamber.

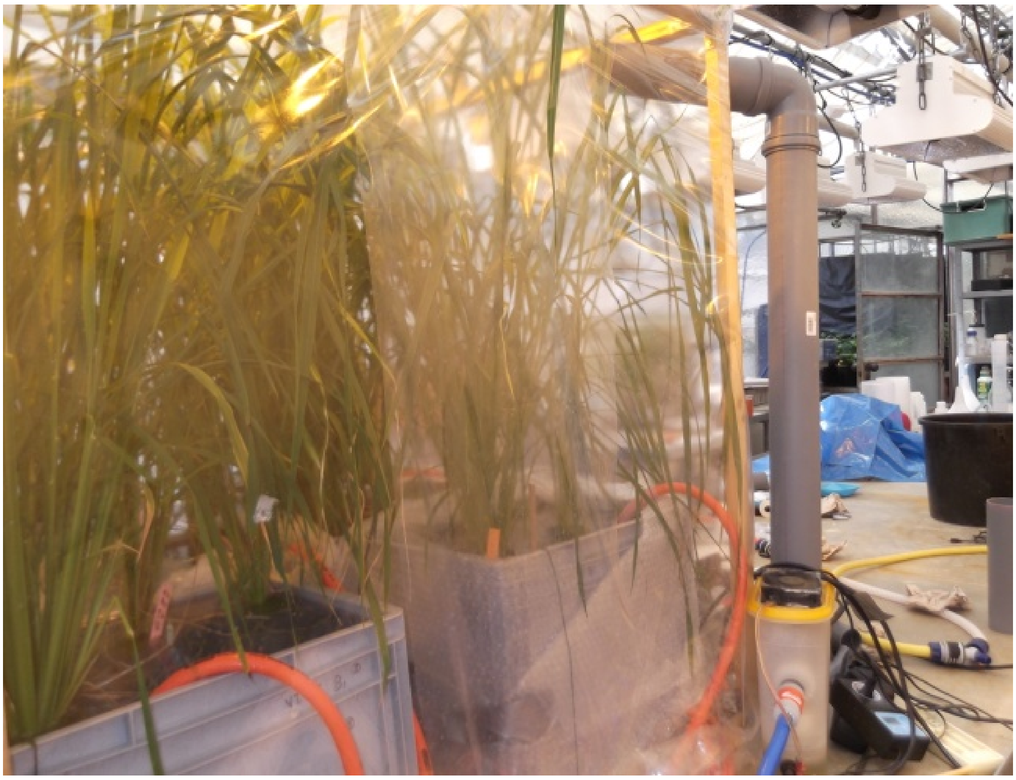

Figure 2: Low VPD Chamber.

- **High VPD**

  This chamber was not supplied with fog and kept open on two opposing sides that air could pass continuously through the chamber as shown in figure 3.

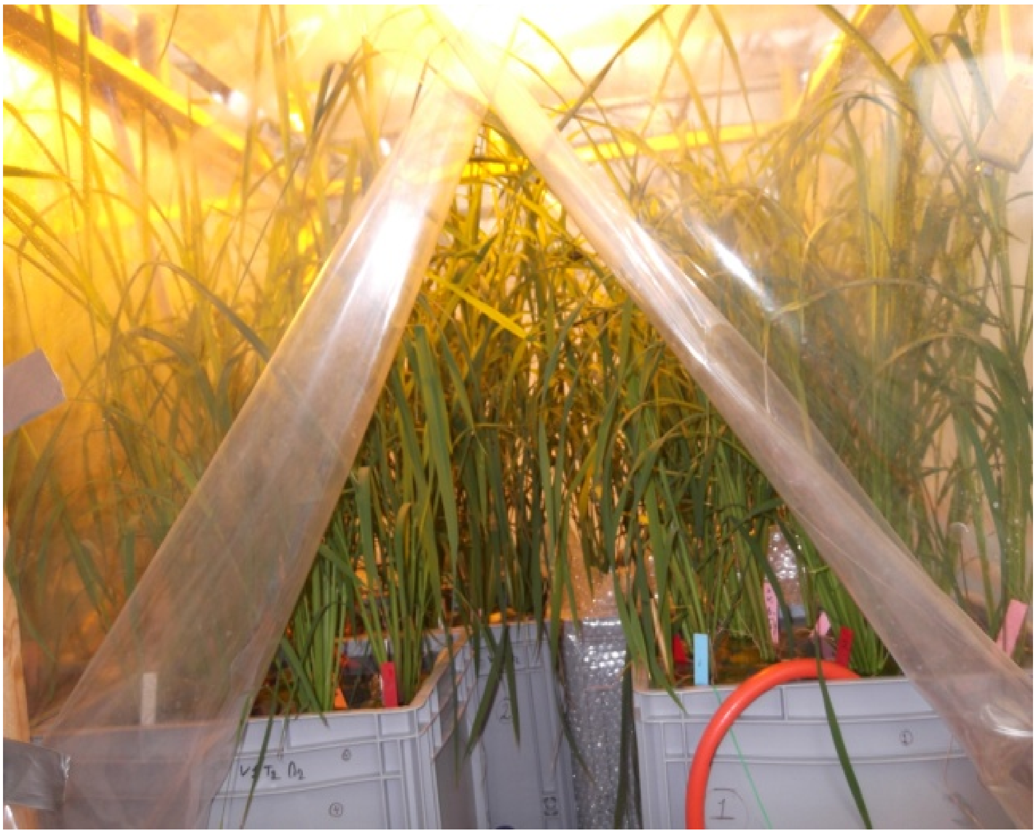

Figure 3: High VPD Chamber.

5. Observation and Measurement

5.1 Temperature and Humidity

Temperature and relative humidity were recorded every minute using Tinytag data loggers (Type TGP-4500, Gemini data loggers, Chischester, UK). Daily average temperature and relative humidity during the experiment is shown in figure 4.

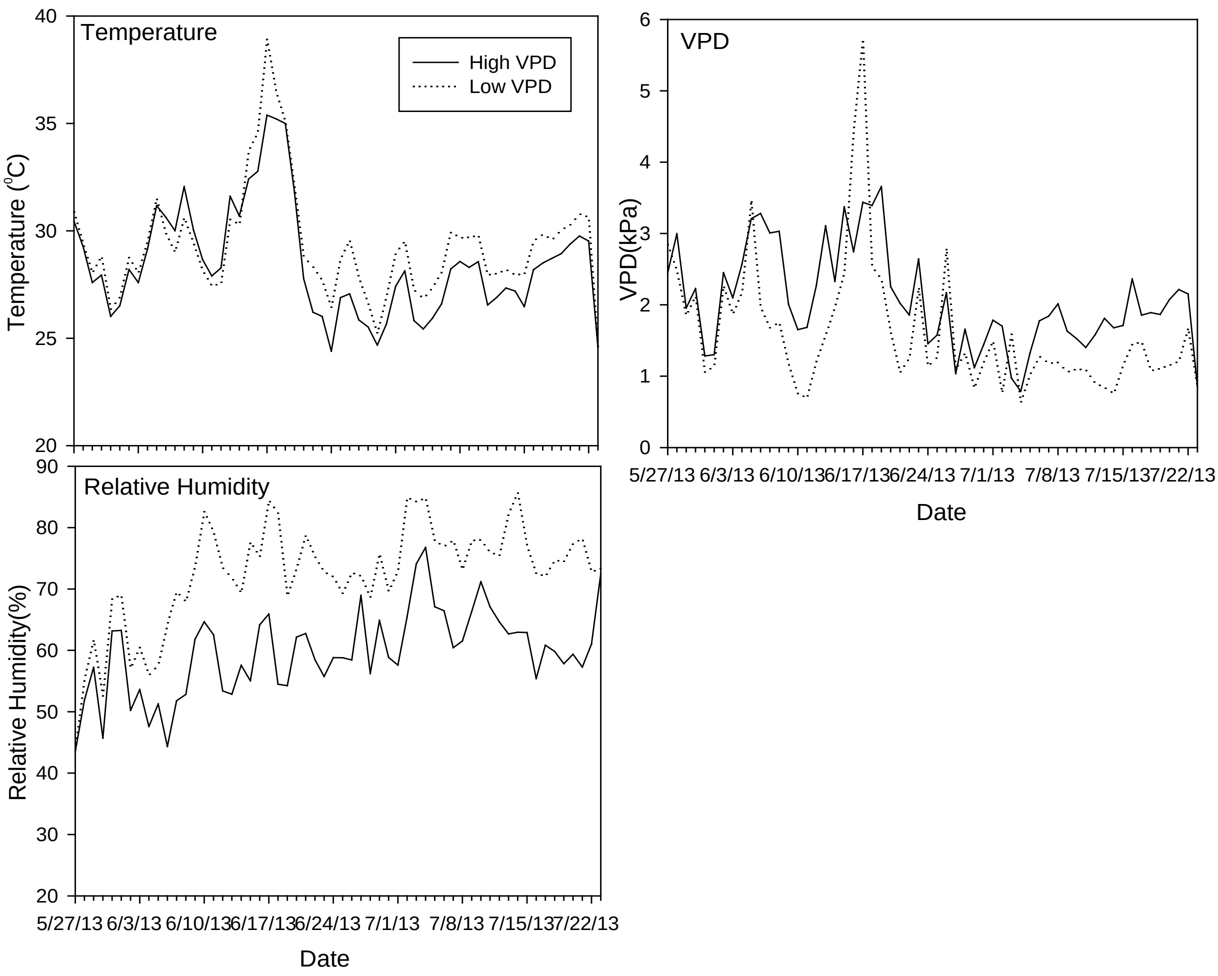

Figure 4: Average temperature (^{0}C) and Humidity (%) recorded by data logger fixed in each chamber.VPD calculated from temperature and humidity data.

Daily temperature of the nutrient solution was recorded every minute using Hobo pendant temperature data loggers (64K-UA-002-64) in both chambers differening in VPD. Daily average temperature of the nutrient solution in three temperature levels and two VPD levels is shown in figure 5.

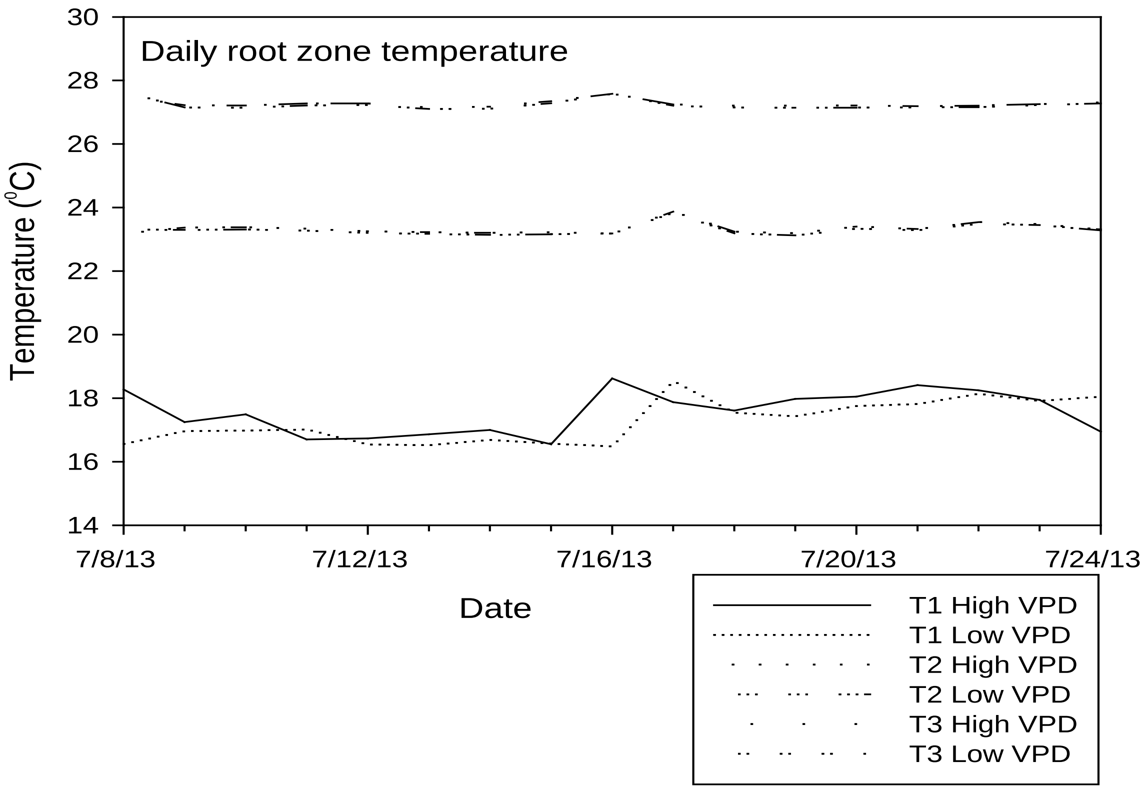

Figure 5: Average daily root zone temperature (^{0}C) recorded by data logger fixed in each threeboxes in each chamber.

5.2 Phyllochron

Phyllochron was observed on the main calm for two varieties in each of the six treatments. Observation started from appearance of the fourth leaf after plants were transferred into the hydroponic system. Observations for new leaf emergence, fully developed leaves on the main culm and dead leaves were done on a daily basis. A leaf was defined as “fully developed” when the ligule was formed. Leaves were marked with a pen. A leaf was defined as “dead leaf” when 50% of the leaf was dead. Dates of each observation were noted and averaged for both varieties.

5.3 Sampling

Six samplings were conducted starting at 17 days after sowing (DAS) and the last at 74 DAS. The first sampling was conducted when the plants were transferred to the hydroponic system. Further five samplings were conducted afterwards in intervals of 10-11 days. During every sampling, 36 plants were harvested in total. Each sampling was done during two days due to the large number of samples.

1st day: Non-destructive measurements were performed. Plants were randomly selected. Leaf number (LN) of the main tiller and tiller number (TN) of each plant were counted. Plant height (PH) and root length (RL) were measured with a folding ruler.

2nd day: Destructive measurements were performed. Same plants observed on the 1st day were harvested. To prevent leaf rolling, detached leaves were scanned for leaf area (LA) using a LI-3100 area meter. All separated leaves, stems and roots were packed in paper bags and dried in the oven for 48 hours at 70^{0}C.

Then 2 days after drying, dry weight were measured separately for leaves, stems and roots in the laboratory by using a weighing machine, also called balance.

5.4 Data processing and problems during research

5.4.1 Data Processing

Absolute growth rate (AGR)

Absolute growth rate (AGR) is the total gain in height or weight by a plant within a specific time interval. It is generally expressed as cm/day in case of plant height and g/day in case of dry matter accumulation per plant. It was calculated with the following formula (Dube, 2011):

AGR (height/day) = H_2-H_1/ t_2-t_1

For our case,

AGR (g/day) = AGBM $_{(54\ DAS)}$ - AGBM $_{(43\ DAS)}$/ t $_{(54\ DAS)}$ - t $_{(43\ DAS)}$

Leaf area expansion rate (LER)

Leaf area is crucial for crop light interception, thus has a substantial influence on crop growth. In order to observe treatment effects on leaf growth, leaf area expansion rate was calculated based on the formula following Dube (2011):

LER (cm^2/day) = $LA_{(54DAS)} – LA_{(43DAS)} / t_{(54DAS)} – t_{(43DAS)}$

5.4.2 Problems during research

- Height of the boxes and the chamber were not suitable which restricted root and shoot development of the plants in the later period of the experiment.
- Due to some technical problems in the greenhouse, sometime shut down of power supply resulted in insufficient nutrient supply during the night.
- Difficulties to maintain constant root zone temperatures were faced. Especially during hot days, it was difficult to maintain the lowest temperature level, which was then sometimes similar to the medium temperature level. Since the temperature in the greenhouse could be hardly controlled on hot days, this effect could not be avoided.

5.5 Statistics

Data obtained in the experiment were entered in excel spreadsheets and statistically analyzed using a least significance difference (lsd) t-test with SPSS. Graphs were generated with the help of sigma plot.

6. Results

6.1 Phyllochron

Phyllochron and leaf development are shown in figure 6 and 7 for three water temperature treatments under high and low VPD for IR64 and Chomrong, respectively. Plants were observed starting from 15 DAS, after being transferred into the hydroponic system when they were at 3-4 leaf stage.

The total number of leaves until maturity varied among the varieties and was influenced by the temperature treatment, but only Chomrong displayed noticeable difference between VPD treatments under high temperature. In total, 14 (IR64) leaves under medium and high temperature under both VPD conditions and 14(Chomrong) leaves under high temperature (low VPD) and 12 (high VPD) appeared on the main culm. At low temperature, 12(IR64) and 10(Chomrong) leaves appeared under both VPD conditions.

Appearance from the 5th leaf was delayed by maximum 10 (IR64) and 14 days (Chomrong) under low temperature compared to medium and high temperature. The effect of low temperature was more pronounced starting from 9th (IR64) and 6th (Chomrong) leaf. No markedly difference between medium and high temperature was detected until appearance of the 8th (IR64) and 6th (Chomrong) leaf, afterwards leaves appeared maximum 8 (IR64) and 10 (Chomrong) days earlier under high temperature compare to medium temperature. Appearance of leaves was not much affected by VPD except for some leaves. In general, most of the leaves appeared 1-2 days earlier under low VPD (IR64), whereas in Chomrong most of leaves appeared 1-2 days earlier under high VPD except for the plants in the high temperature treatment.

The total number of dead leaves until maturity varied among the varieties and was influenced by the temperature treatments with only minor influence of VPD. Only 2 (IR64) and 4 (Chomrong) leaves died under low temperature, whereas up to 7 (IR64) and 8 (Chomrong) leaves died under medium and high temperature. Leaves with the same number displayed a strong increased senescence rate in the medium and high temperature compared to the low temperature. Leaves of both varieties survived up to 25 days longer under low temperature. In IR64, most of the leaves died earlier under low VPD and the effect was stronger under high temperature (up to 10 days), whereas in Chomrong, contrasting results were observed. There, almost all leaves died earlier under high VPD (up to 13 days) except leaves in low temperature died earlier (up to 18 days) under low VPD.

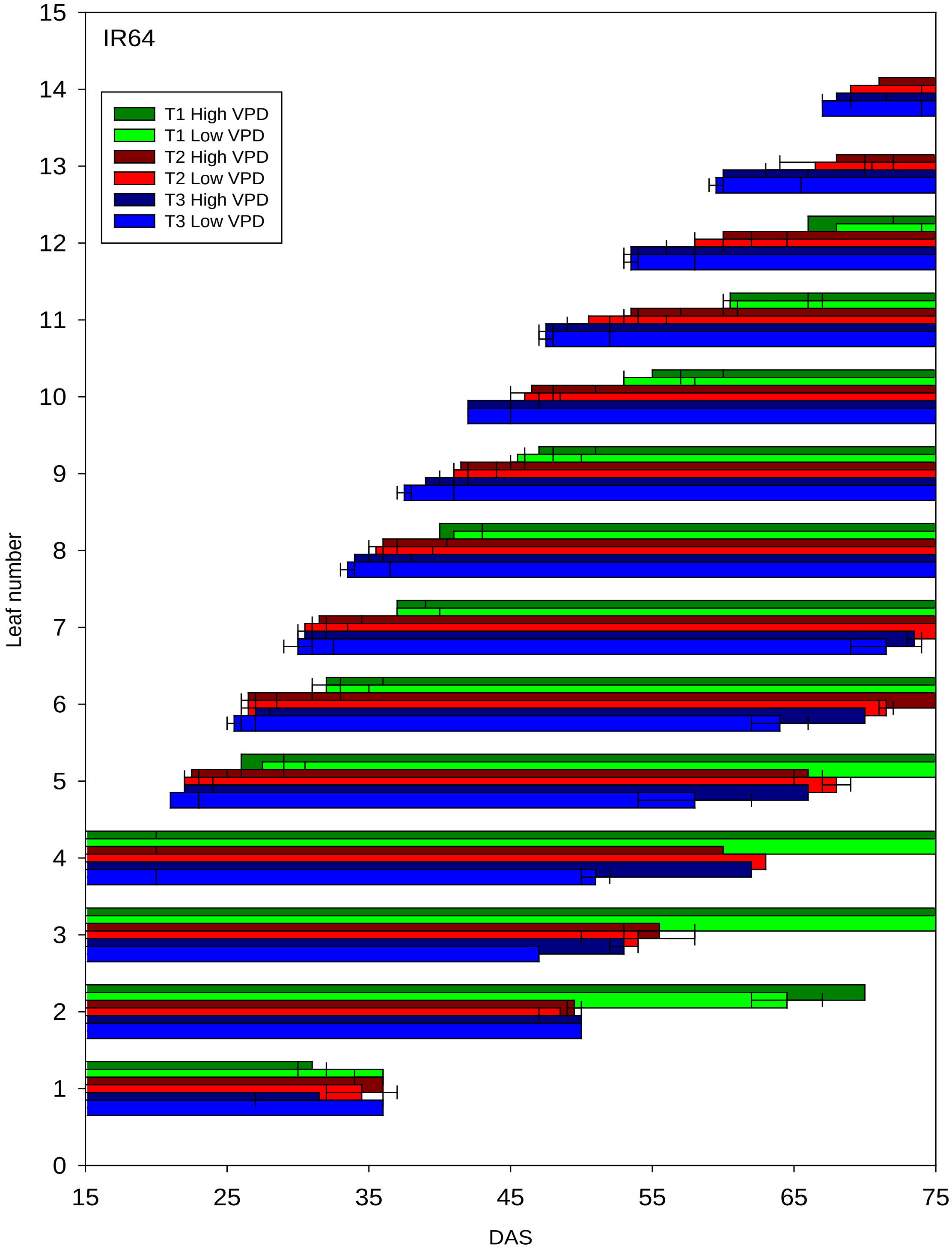

Figure 6: Leaf Development of IR64 under three water temperatures,T1=Low (green), T2= Medium (red) and T3= High (blue) and two VPDs, High (dark) and Low (light). Leaf appearance =beginning of the bar, fully developed= vertical line inside, leaf death=end of the bar, leaf duration=whole length. Error bars indicate standard errors.

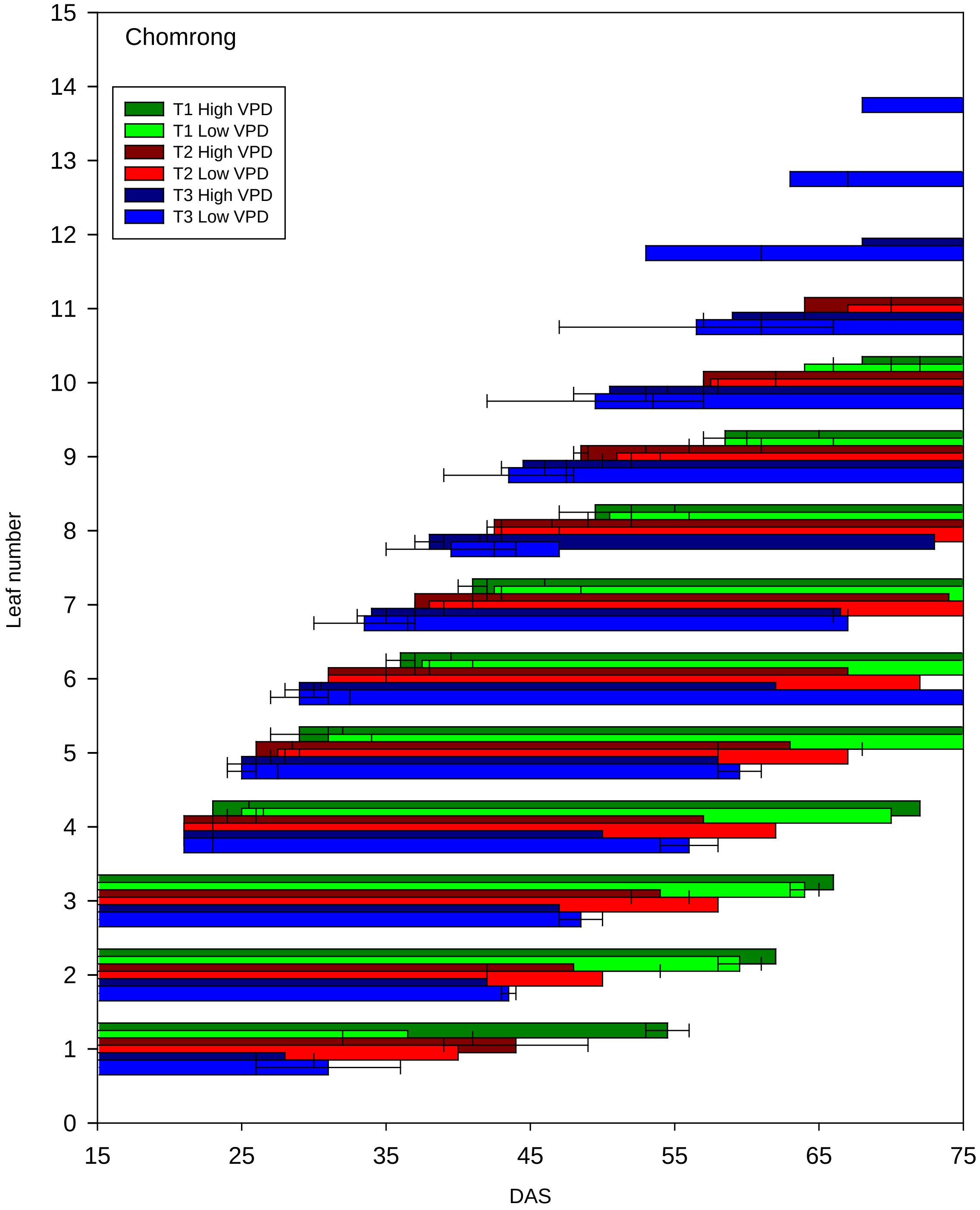

Figure 7: Leaf Development of Chomrong under three water temperatures,T1=Low (green), T2= Medium (red) and T3= High (blue) and two VPDs, High (dark) and Low (light). Leaf appearance =beginning of the bar, fully developed= vertical line inside, leaf death=end of the bar, leaf duration=whole length. Error bars indicate standard errors.

Phyllochron varied between the temperature treatments with no significant influence of VPD treatment. In general, phyllochron under low temperature was larger (up to 7 days) whereas under medium and high temperature it was up to 5 days for earlier leaves which increased for later leaves.

Total leaf duration (TLD) varied with treatments. Under medium and high temperature, total leaf duration was significantly shorter compared to low temperature for both varieties. Small differences were observed in TLD under different VPD condition. In most cases, TLD was increased under low VPD (in medium and high temperature), but under low temperature, it was decreased under low VPD for both varieties.

In Chomrong, at high temperature and low VPD condition, 8th leaf died after 10 days of emergence because the 8th leaf was infected when it emerged, showing yellowish colour which did not survive longer and died after 10 days. Also, 12th leaves appeared earlier than 11th leaf, which is not true. This is due to the different trend of leaf emergence between the replicates. Up to 11th leaf emerged in one replicate, whereas up to 14th leaf emerged in another replicate. While doing the average of replicates at 11th leaf, it seems 12th leaf emerged earlier than the 11th leaf.

6.2 Morphology

6.2.1 Plant height and Tiller number

Average plant height and tiller number are shown in figure 8 and 9 for two varieties in different water temperature and VPD treatment at different sampling dates, respectively. Plant height and tiller number were significantly different between temperature treatments, about only small differences were found between high and medium temperature. No large difference across VPD treatment was observed except for minor differences.

Maximum plant height was found for IR64 under high temperature (102 cm), whereas in Chomrong it was found under medium temperature (138 cm). Under low temperature, plant height was up to 40% lower compared to the other two temperatures treatments. Under low VPD, after 54DAS plant height remained constant or decreased in some case at high and medium temperature treatments.

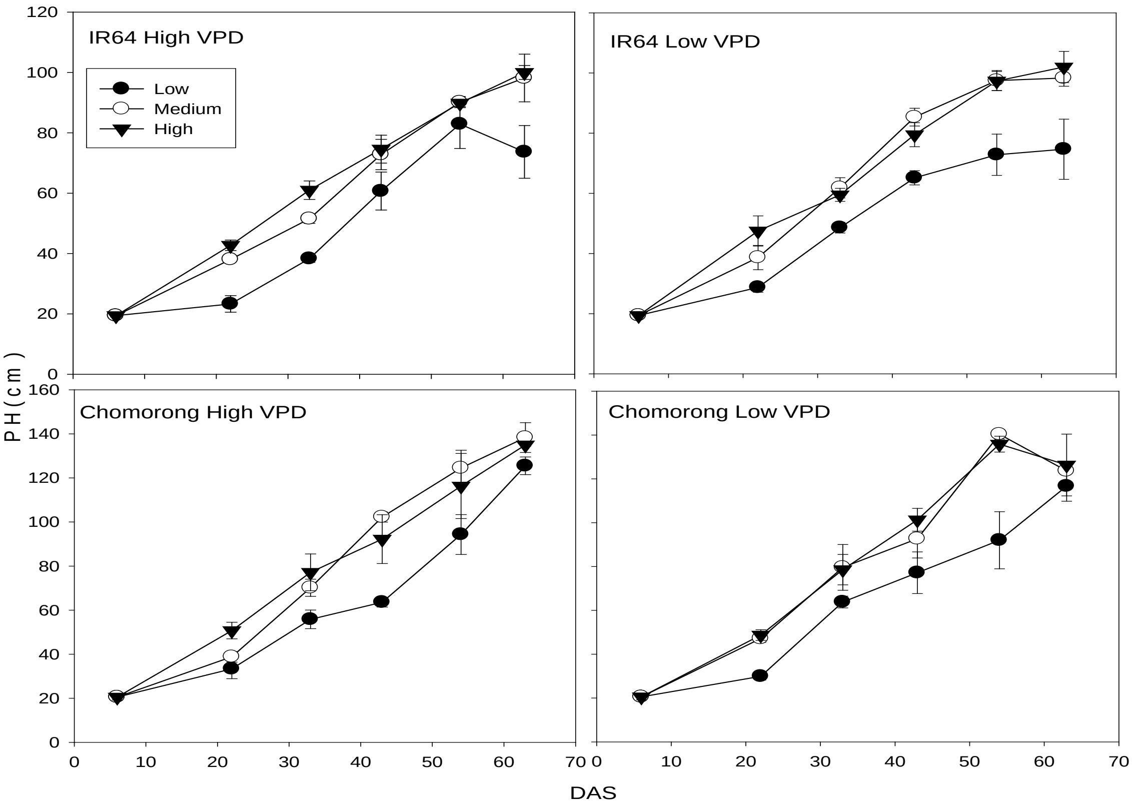

Figure 8: Average plant heights (cm) of IR64 and Chomrong at each sampling date for three water temperature treatments under high and low VPD conditions. Error bars= Standard error of mean.

The number of tillers was higher (up to 34) in IR64 than in Chomrong (up to 20). Number of tillers was highly reduced under low temperature (up to 50-60%) and the effect of temperature was stronger under low VPD for both varieties. Interestingly, for IR64 under high VPD, more tillers were found under high root zone temperature, whereas under low VPD, medium temperature resulted in the highest number of tillers.

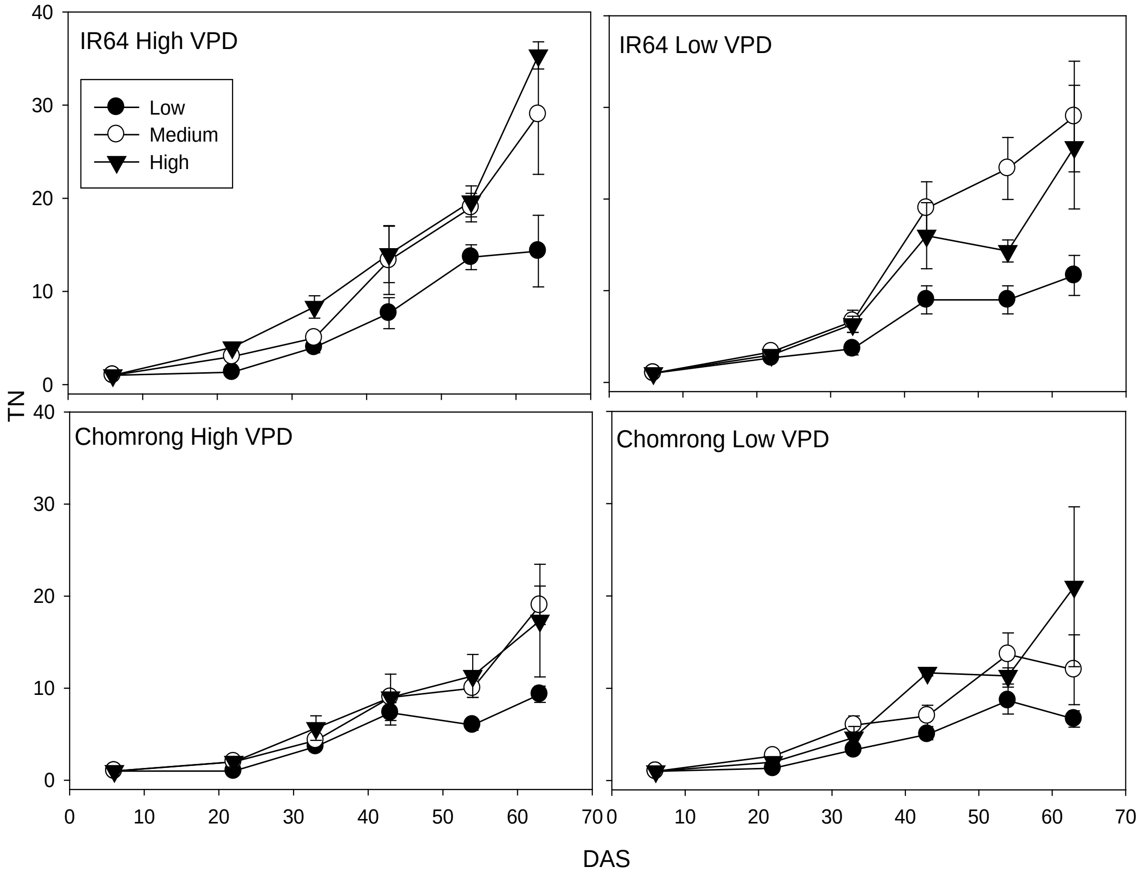

Figure 9: Average Tiller numbers of IR64 and Chomrong at each sampling date for three temperature treatment under high and low VPD conditions. Error bars= Standard error of mean.

6.2.2 Above ground biomass and Root dry mass

AGBM was maximum 22 g (IR64) and 19 g (Chomrong) at 64 DAS under high temperature. For both varieties, AGBM was significantly lower under low temperature, whereas small differences between medium and high temperature were found. In general, AGBM was comparatively lower under low VPD in all temperature treatments for both varieties with some exceptions.

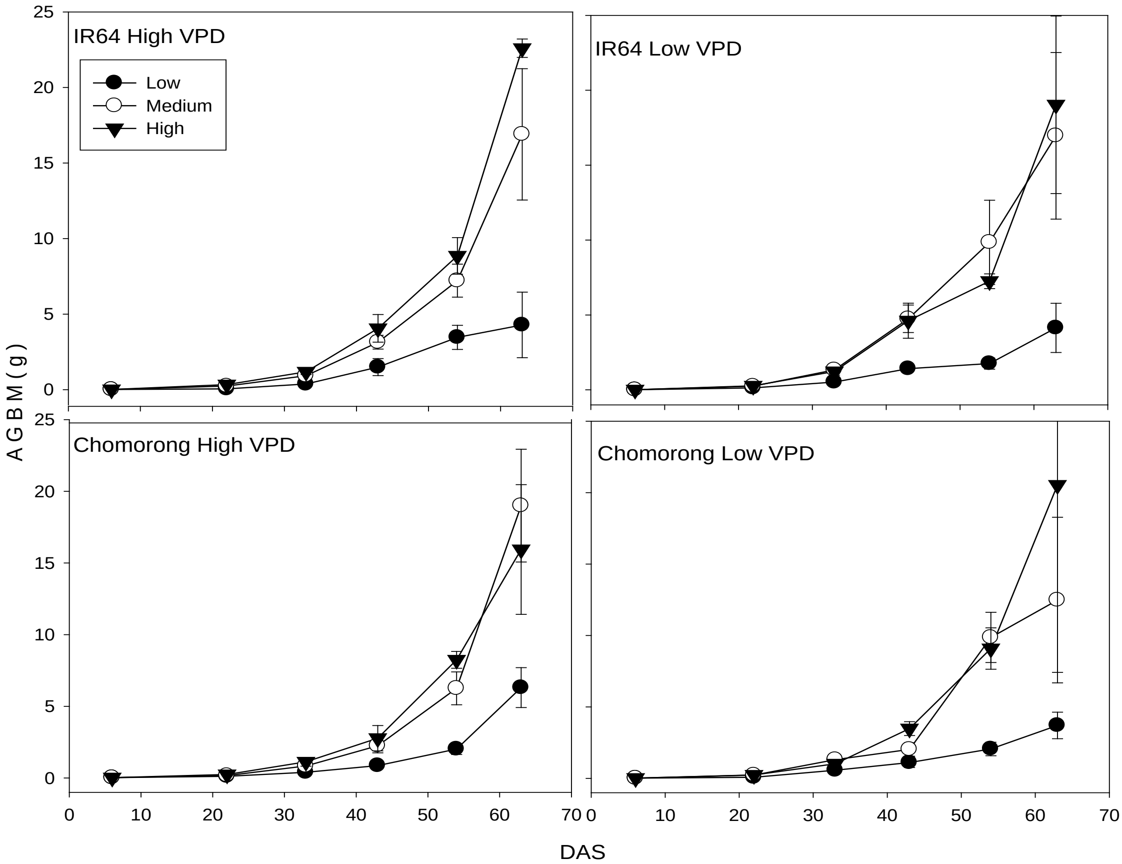

Figure 10: Average Above ground biomass (g) of IR64 and Chomrong at each sampling date for three temperature treatment under high and low VPD conditions. Error bars= Standard error of mean.

RDM was maximum at 64 DAS with 5.8 g (IR64) and 4.3 g (Chomrong) under high and medium temperature respectively and these values were observed under high VPD conditions. RDM was significantly lower under low temperature and the difference between high and low temperature was larger in IR64 than in Chomrong. This difference was smaller in IR64 and larger in Chomrong under low VPD condition. In IR64, no differences under low temperature between VPD treatments was found, whereas in Chomrong RDM was lower under low VPD.

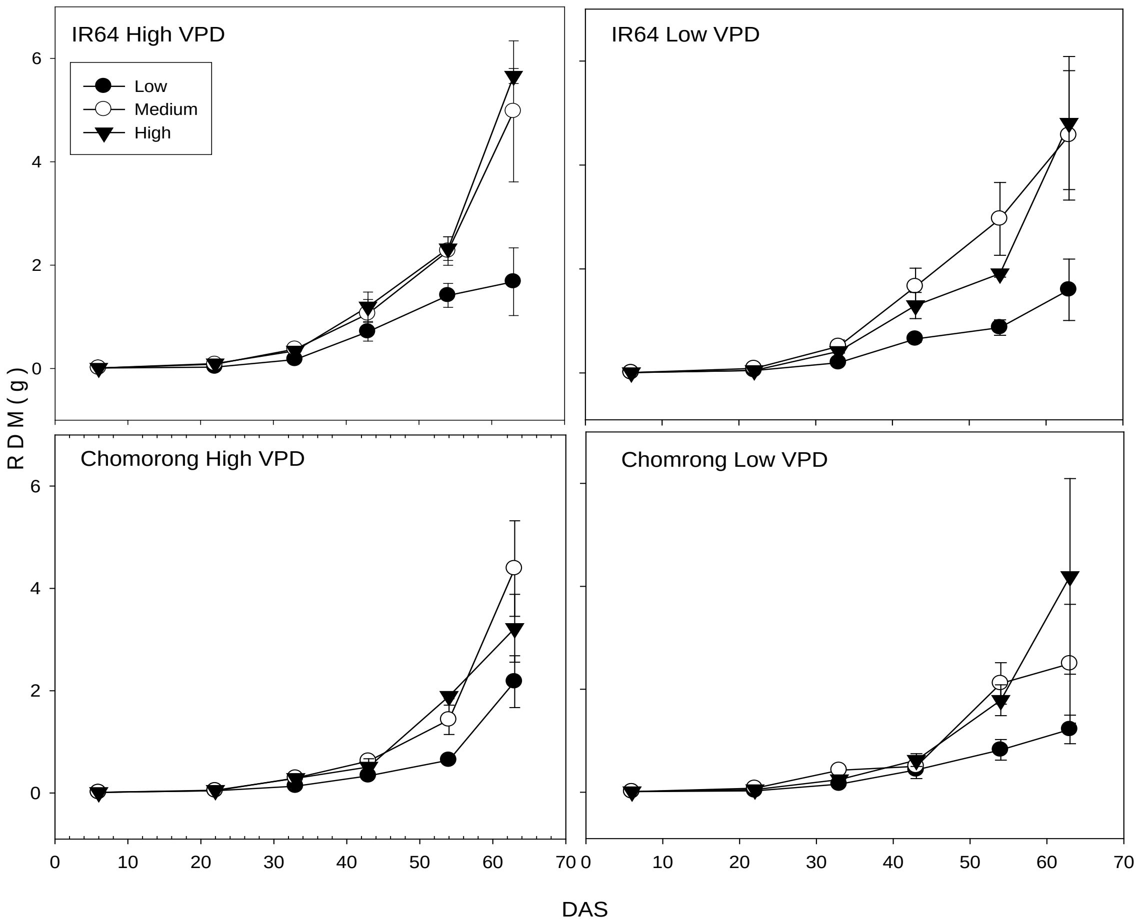

Figure 11: Average Root dry mass (g) of IR64 and Chomrong at each sampling date for three temperature treatment under high and low VPD conditions. Error bars= Standard error of mean.

6.2.3 Leaf area and leaf area per tiller

Under high temperature, maximum LA was reached at 64 DAS with 4024 cm^2 (IR64) and 1426 cm^2 (Chomrong) for both varieties, LA was lowest under low temperature. At low temperature, LA was comparatively higher under low VPD up to 33 DAS then decreased in later sampling date for both verities. At high temperature, LA was lower (IR64) and higher (Chomrong) under low VPD condition in most sampling date. At medium temperature, LA was more under low VPD for both verities in most sampling date.

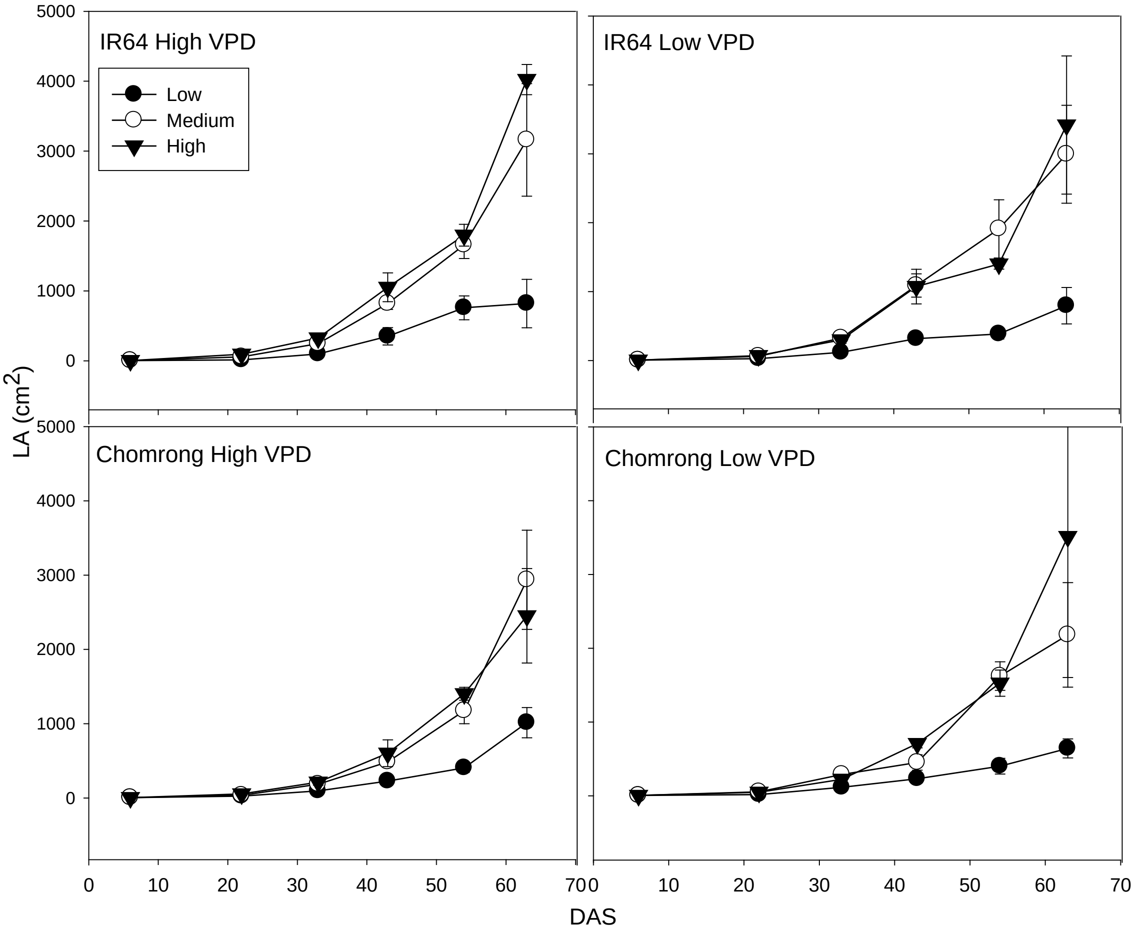

Figure 12: Average Leaf area (cm2) of IR64 and Chomrong at each sampling date for three root zone temperature treatments under high and low VPD conditions. Error bars= Standard error of mean.

LA/T was lowest under low temperature. In general, under higher temperature LA/T was higher than under medium temperature across VPD for both varieties in most of the sampling dates. In most cases, at low temperature, LA/T was higher under high VPD for both varieties. At medium temperature, LA/T was higher in IR64 and lower in Chomrong under conditions of high VPD. At high temperature, LA/T was higher (IR64) and lower (Chomrong) under conditions of low VPD in most sampling dates.

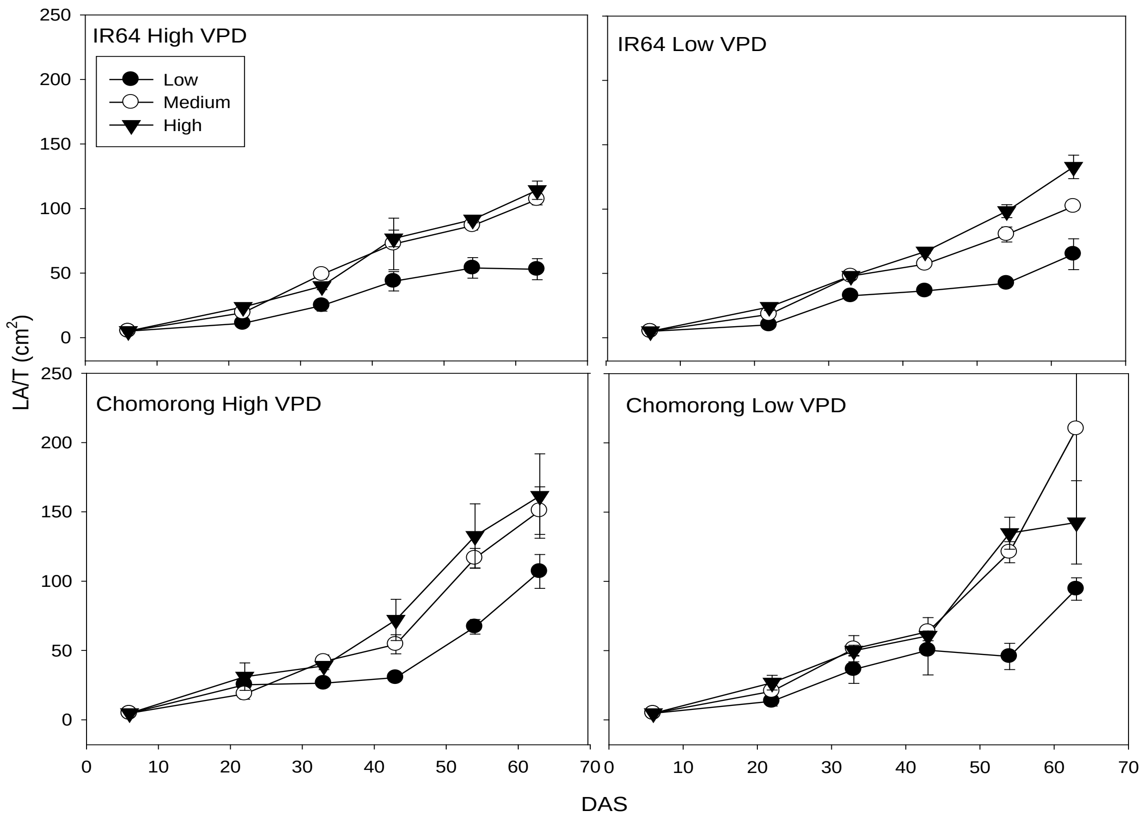

Figure 13: Average leaf area per tiller (cm2) of IR64 and Chomrong at each sampling date for three temperature treatments under high and low VPD conditions. Error bars= Standard error of mean.

6.2.4 Temperature treatment specific comparison of plant parameters at 54 DAS

In total, 6 samplings were conducted for both varieties in the three root zone temperature treatments: at 6 DAS, 22 DAS, 33 DAS, 43 DAS, 54 DAS, and 63 DAS respectively. Among these sampling dates, sampling at 54 DAS was selected for comparison of plant morphology (figure 14), since to this point, large differences between treatments could be observed and growth was not yet limited by the size of the chamber as it was the case at 63 DAS. Plant height (cm), tiller number, aboveground biomass (g), root dry mass (g), leaf area (cm^2) and leaf area per tiller (cm^2) were recorded. Most of the recorded plant parameters were significantly lower under low temperature.

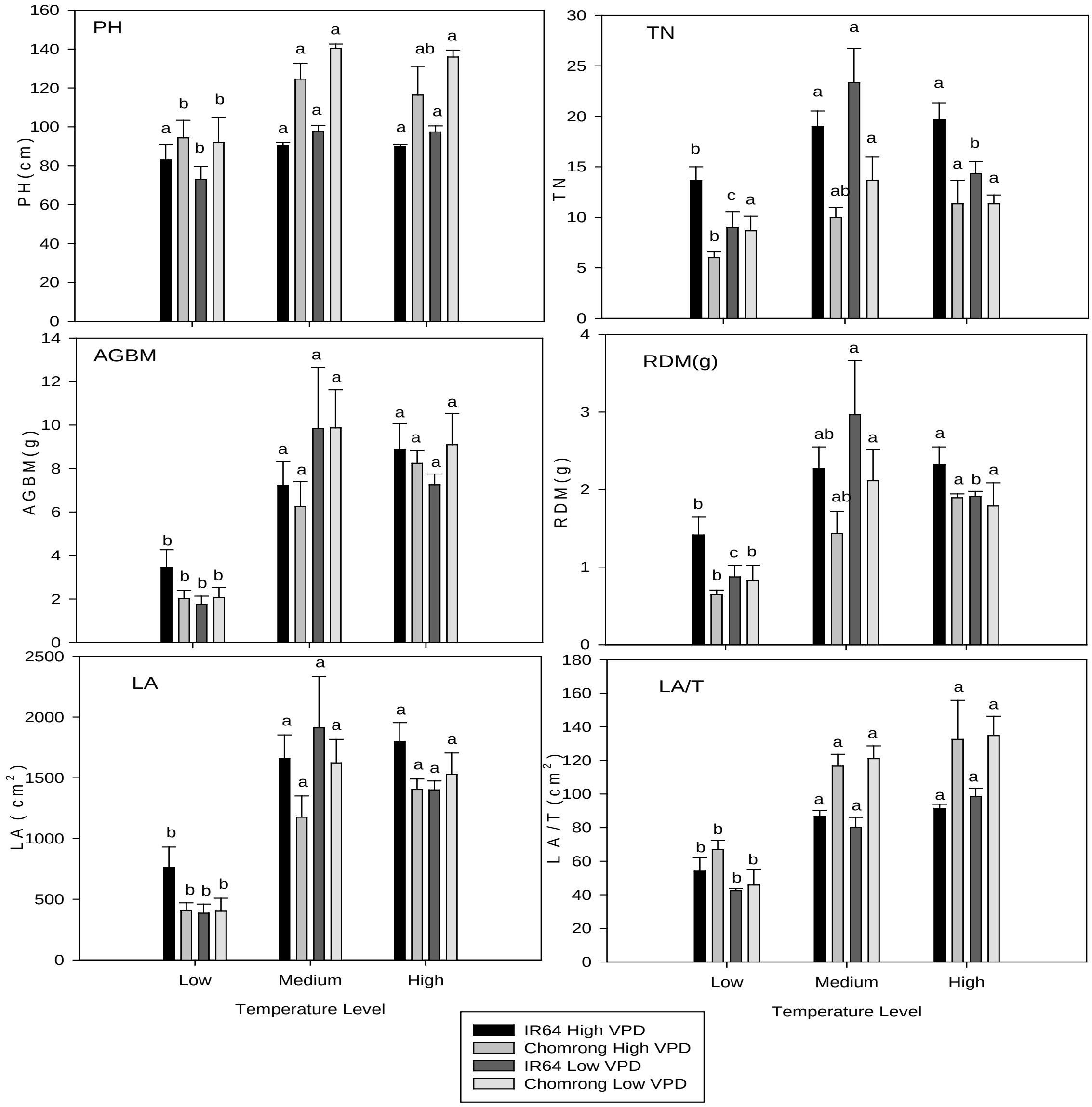

Figure 14: Average PH=plant height (cm), TN=tiller number, AGBM=above ground biomass (g), RDM=root dry mass (g), LA=leaf area (cm2), LA/T=leaf area per tiller (cm2) at 54 DAS (4th sampling) for three root zone temperature treatments under high and low VPD conditions, Error bars= SE of means. Means with same letter are not significantly different between temperature treatments; p≤0.05.

Plant height was significantly lower at low temperature except for IR64 under high VPD. As the only variety, IR64 showed no change in height across temperature treatments. There was no significant difference between medium and high temperature except for Chomrong under high VPD, which was higher under medium temperature. At LRT, IR64 was significantly shorter under low

VPD compared to high VPD. At high temperature, Chomrong was significantly higher under low VPD than under high VPD.

Tiller number was significantly lower under low temperature except for Chomrong, which did not show any significant differences in tiller number between temperature treatments under low VPD. IR64 had significantly more tillers under low VPD under medium temperature than under high temperature. At low temperature, a significant difference between VPD treatments was observed for both varieties. Here, more tillers were observed in IR64 under high VPD, whereas low VPD resulted in more tillers in Chomrong.

Aboveground biomass, Leaf area and leaf area per tiller were significantly lower under low temperature, but no significant differences were found between medium and high temperature for both varieties across VPD treatments.

Root dry mass was significantly low at low temperature for both varieties under both VPD treatments. IR64 at low VPD had a significantly higher root dry mass under medium temperature compared to high temperature. At low temperature, IR64 showed significantly more root dry mass under high VPD, whereas Chomrong did not show any significant difference between VPD treatments.

6.3 Absolute growth rate and Leaf Area expansion rate

Absolute growth rate (AGR) and leaf area expansion rate (LER) between 43 DAS and 54 DAS are shown in figure 15. Both increment parameters varied among treatments and varieties.

AGR was significantly reduced (by almost 100%) at low temperature except for IR64 at high VPD, which did not differ between temperature treatments. AGR was not significantly affected by VPD across temperature levels in most cases. At low temperature, IR64 showed significantly lower AGR under low VPD, whereas Chomrong showed no significant differences between VPD levels. At medium temperature, AGR was higher at low VPD for both varieties, whereas at high temperature, it decreased for IR64 and chomrong showed no differences.

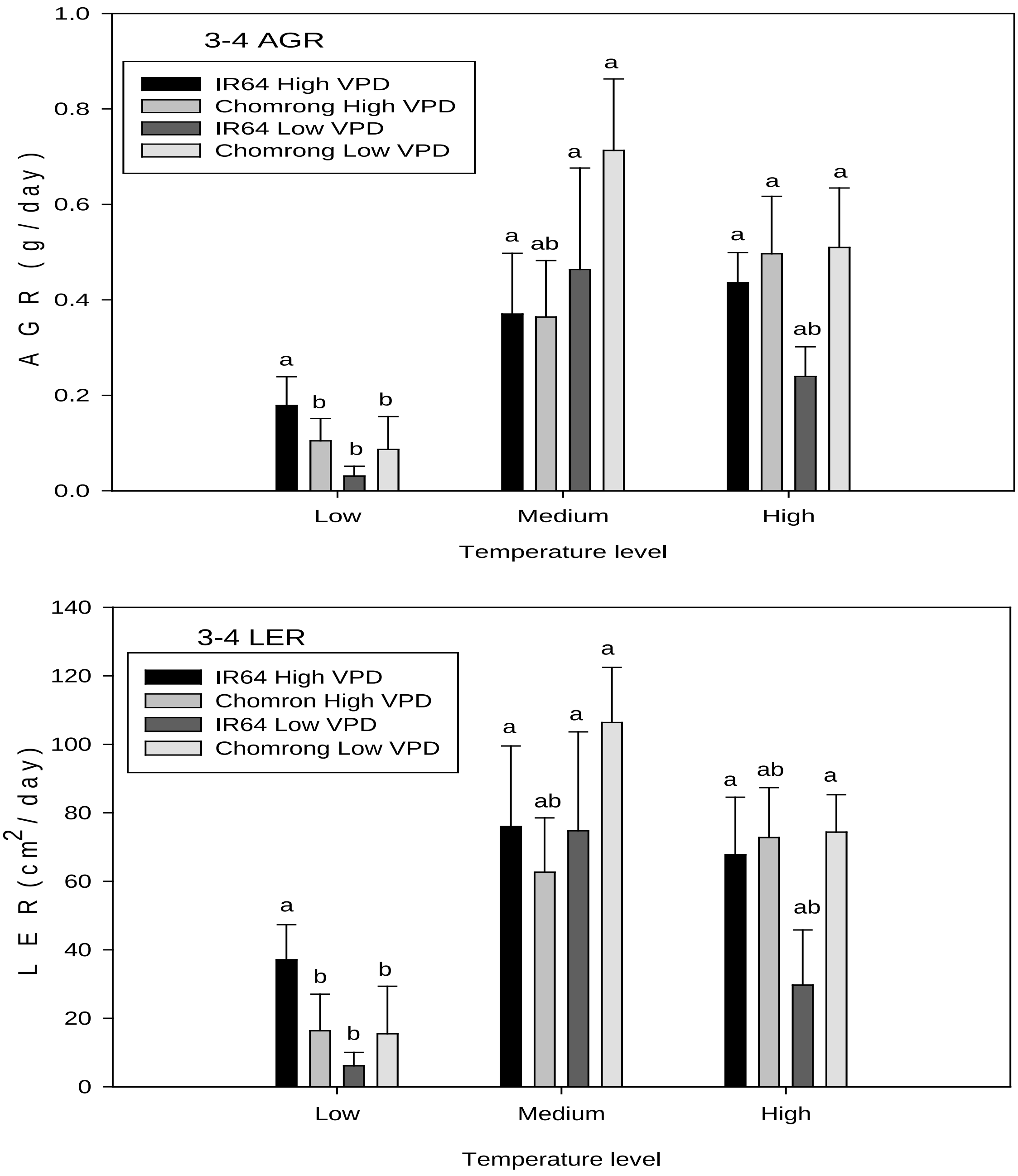

Figure 15: AGR=absolute growth rate (g/day) and LER=leaf area expansion rate (cm2/day) between 43 DAS (3rd sampling) and 54 DAS (4th sampling) for three temperature treatment under high and low VPD condition, Error bars= SE of means. Means with same letter are not significantly different between the temperature; p≤0.05.

Leaf area expansion rate (LER) was decreased by more than 50% at low temperature except for IR64 at high VPD, which did not show differences between temperature levels. There was no significant difference in LER between medium and high temperature, although under medium temperature LER was higher for Chomrong. A significant effect of VPD was observed at low and high temperature for IR64, where LER was markedly declined under low VPD. Chomrong at medium temperature displayed increase in LER at low VPD.

6.4 Biomass Allocation

Both varieties displayed significant reduction in leaf mass fraction (LMF=LDW/TDW) when exposed to low temperature, whereas no significant differences between medium and high temperature was found. LMF was approximately doubled in medium or high temperature compared to the low temperature treatment. Despite minor differences, no significant differences between VPD treatments were observed in all temperature for both verities. Generally, more leaf biomass allocated under high VPD except at high temperature where more under low VPD.

Root mass fraction (RMF=RDW/TDW) strongly increased under low temperature whereas no significant difference between medium and high temperature for both verities. There was no markedly difference in RMF among different temperature level at different VPD for both verities. In general, at low temperature, both verities displayed higher RMF at low VPD.

Stem mass fraction (SMF=SDW/TDW) declined significantly when exposed to low temperature except IR64 at high VPD remain unchanged under all temperature level. There was no significant difference among VPD treatment at different temperature level except IR64 at low temperature where, SMF decreased significantly under low VPD.

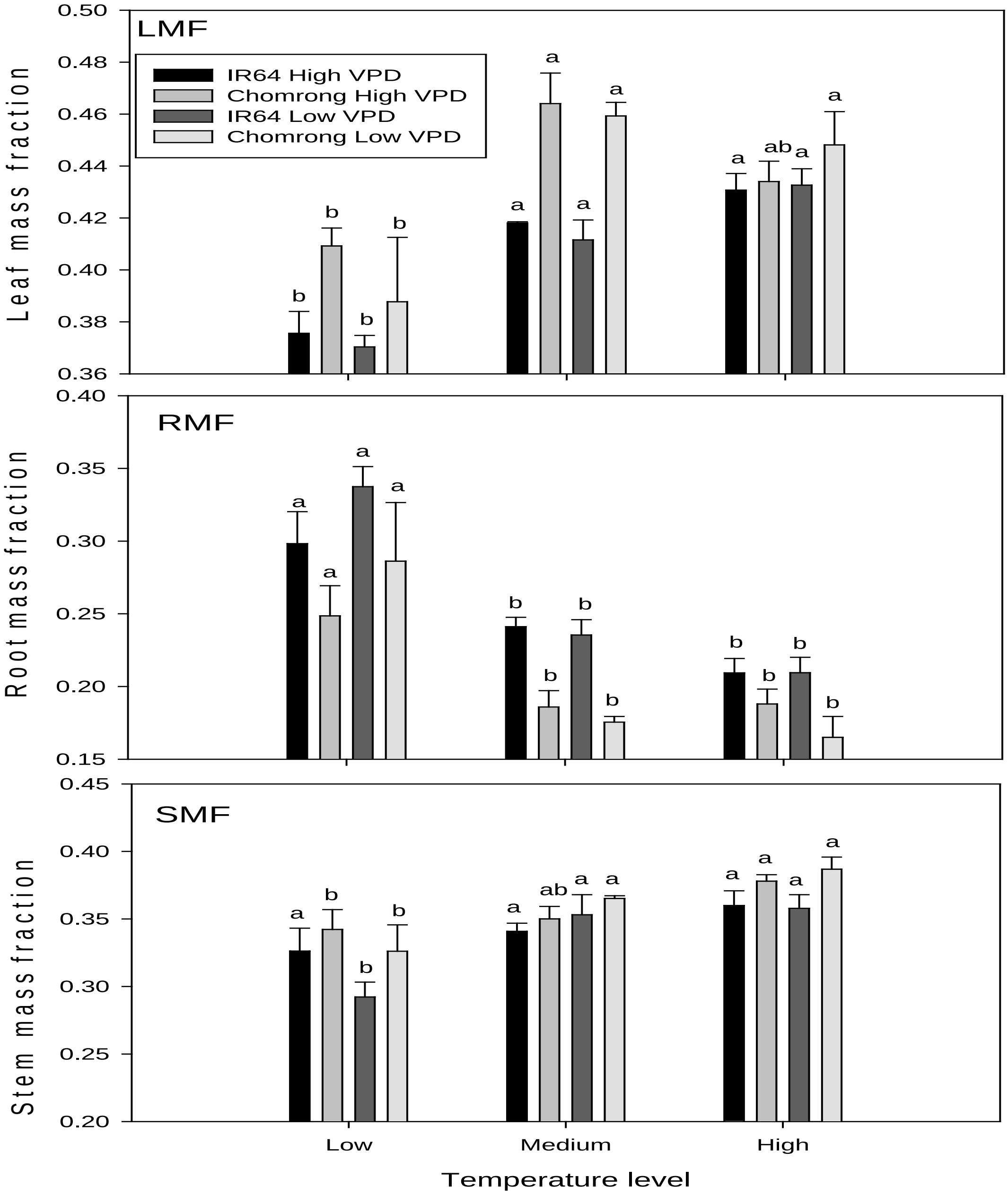

Figure 16: LMF=leaf mass fraction, RMF=root mass fraction, SMF=stem mass fraction at 54 DAS (4th sampling) for three temperature treatment under high and low VPD condition, Error bars= SE of means. Means with same letter are not significantly different between the temperature; p≤0.05.

7. Discussion

7.1 Temperature and VPD treatment

Daily average root zone temperatures (^{0}C) of all three temperature treatments are shown in figure 5. No data is shown for the first few weeks due to a technical problem. The root zone temperature remained similar in both chambers till the end of the experiment, in the medium and high temperature treatments. On average, medium and high temperature treatments were 23.2 ^{0}C and 27.7 ^{0}C, respectively. There were only minor differences between the chambers in the low root zone temperature treatment. In most cases, LRT was slightly higher under high VPD conditions. On average, LRT was 17 ^{0}C.

Daily average temperature (^{0}C), humidity (%) and calculated VPD (kPa) of each chamber are shown in figure 4. The daily average temperature was almost similar in both chambers during the first few weeks, while the temperature was slightly higher in the low VPD chamber afterwards. The VPD level difference between the chambers was not observed as expected. Nevertheless, in most of the dates the VPD level was lower in the low VPD chamber.

7.2 Effects on Leaf appearance rate

One of the objectives of this study was to assess the effect of root temperature on Leaf appearance rate (LAR) of rice, since leaves are the primary organs for dry matter production and yield via photosynthesis. Despite many findings regarding LRT attributed water uptake inhibition (e.g. Kuwagata et al., 2004; Murai-Hatano et al., 2008), temperature at meristem and its effects on LAR have been rarely considered in those studies. Air and water temperature influence the Growth and development of rice crop (Dingkuhn et al., 1995), particularly for irrigated rice thermal environment of the growing point should be considered for crop ontogeny studies because shoot meristem is exposed to water temperature until booting stage. Yin et al. (1996) reported that meristem temperature control the LAR. Ellis et al. (1993) found delayed LAR at 20 ^{0}C compared to 24^{0}C and 28^{0}C temperature. Although all the plants grew under the same air temperature, LAR increased with increasing root temperature up to

T_{opt} 27.7^{0}C. As the meristem was inside the nutrient solution indicating that meristem temperature mainly attributed to changes in LAR rather than air temperature.

In wheat, leaf appearance comprised with cell division and cell expansion at meristem, and cell division was controlled by temperature (McMaster et al., 2003). Therefore, it can be reasoned that slow leaf appearance at low meristem temperature could be attributed to inhibited division. Although there is considerable experimental evidence and theoretical reasoning that soil temperature at meristem is more influential than air temperature, some limited evidence suggests that this hypothesis may be too simplistic.

Phyllochron was delayed under LRT, agreeing with Itoh and Sano (2006), who stated that the time necessary to form each phytomer was longer at low temperature conditions. Total number of leaves was markedly lower at LRT is caused by delayed LAR.

High VPD reduces stomatal conductance caused by stomatal closure mediated by high transpiration demand and thus a decrease in leaf water potential (Bunce, 2006). So far, effects of VPD on leaf appearance rate under controlled root temperature in rice have rarely been studied. Hirai et al. (2000) reported that the function of increased rate of leaf emergence under high humidity (90%) during the dark period, probably result of increased water contents in the plants and hence achieve sufficient available water in the growing point. However, rate of leaf emergence was not affected by high humidity given during both the light and dark period which fits with our findings.

At high temperature, Chomrong showed more number of leaves under low VPD whereas IR46 showed no differences between VPD levels, demonstrating that Chomrong prefers humid conditions which might be linked with origin from high altitude.

At LRT, leaf appearance was delayed more in Chomrong (14days) than in IR64 (10days). Thus the number of leaves on the main column was lower in Chomrong (10) than in IR64 (12). This difference might be caused by the different sizes of the leaves. Leaf size determines the leaf angle, short leaves tends to be more erect and usually more evenly distributed throughout the canopy, which permits less mutual shading of leaves and more efficient use of light for photosynthesis compared to longer leaves (Fageria, 2007). At the same time, Birch et al. (1998) reported that leaf appearance depends on current photosynthesis to meet the demand of growth. Therefore, delayed leaf appearance in Chomrong might be caused by bigger leaves compared to the IR64. The number of dead leaves were higher in Chomrong (4) than in IR64 (2) at LRT. This is due to the bigger leaves of Chomrong, which can easily reach the surface, so they are more susceptible to damage by water.

7.3 Effects on Morphology

Our second objective was to analyse morphological responses. Under LRT, above ground biomass (AGBM) significantly decreased and so did leaf number (LN), tiller number (TN) and leaf area (LA) (figure 6, 7, 14). Shimono et al. (2002) found decreased biomass production caused by reduction in crop growth rate, and growth reduction was more during vegetative period when plant meristems are inside the water and less during reproductive period when meristems emerged above the water, indicating that meristem temperature is mainly responsible for biomass production. On the other hand, although plant meristems lifted above the water surface, Nagasuga et al. (2011) found low biomass under LRT due to decreased leaf area, which is associated with an insufficient water supply from root to shoot leading to reduction in water potentials of the stem and growing parts of leaves. Recently Ahamed et al. (2012) found acclimation to LRT after 5 days of treatment by acquiring water uptake mechanism. Since plant roots were chilled more than 5 days in our experiment, the main factor responsible for decreased AGBM is low meristem temperatures (i.e. delayed LAR leading less LN and thus, less LA) rather than the root temperature.

Arai-Sanoh et al. (2010) observed increased weight of shoot and root at 25^{0}C and 32 ^{0}C whereas decreased weight at 18^{0}C and 37^{0}C soil temperature, indicating that optimum temperature for root and shoot growth of rice is 25-32 ^{0}C, which fits with our findings. We found increased shoot and root biomass under 23.2 ^{0}C and 27.7 ^{0}C (figure 10, 11). In their experiment, decline in shoot and root dry weight was related to suppressed photosynthesis, either high ABA leading to reduction in diffusive conductance of leaves under high temperature or inhibited water and nutrient uptake caused by lower root activity and a smaller root zone under low temperature.

To date, very few researches investigate integrated effects of LRT and VPD on plant growth and morphology in rice. Some authors have reported that air humidity in the shoot zone determines the effect of water, salt and temperature stresses (Capell and Dörffling, 1989; Lauter and Munns, 1987; Mizrahi et al., 1971). The feedforward hypothesis assumes that stomatal conductance declines as VPD rises, which helps to minimize transpirational water loss by plants (Farquhar, 1978). The other possible mechanism is the feedback response of stomata which assumes that as VPD increases, stomatal conductance decreases due to a direct increase in transpiration (Mott and Parkhurst, 1991; Moteith, 1995). Based on these hypotheses, a larger effect of LRT under high VPD was expected. Kuwagata et al. (2012) found that plants in the low humidity chamber were subjected to relatively high water stress due to high evaporative demand. Furthermore they found up- regulation of aquaporin in both roots and shoots under low humidity condition. Recently, Ahamed et al. (2012) found acclimation to LRT (by acquiring a water uptake mechanism), which is caused by up regulation of aquaporins. In present study, plant parameters (PH, AGBM, LA, RDM) are higher under high VPD and LRT conditions in most cases (figure 14). Therefore, it could be argued that rice plants are more acclimated to LRT under high VPD compared to low VPD. Presumably, linked to up- regulation of aquaporins. On the other hand, Murai-Hatano et al. (2008) reported that activity of aquaporins plays more important role than their abundance in reducing root hydraulic conductivity under LRT. However, the underlying mechanism of VPD and root temperature interaction (i.e. shoot to root signaling under LRT conditions) is still unclear and further investigation regarding aquaporins activities under high VPD and LRT conditions is required.

Since high temperature leads to short crop duration and thus less tillers (Stuerz, 2014), a higher TN in IR64 under medium temperature (low VPD) can be explained by short crop duration under high temperature.

At LRT, varietal differences were observed in PH, TN and RDW between the VPD treatments. IR64 showed significantly higher PH, TN and RDW under high VPD, which might be originates from tropics under low humid conditions whereas Chomrong showed a significantly higher TN under low VPD, which might be originates from high altitude of Nepal.

7.4 Effects on Growth rate

The thermal environment of the growing point should be considered during the crop ontogeny studies, which is important for irrigated rice, commonly grown in flooded conditions from seedling stage until grain filling (Ritchie, 1993). Shimono et al. (2002) found reduced crop growth rate under low water temperature, more severe during the vegetative period and less during the mid-reproductive period after the meristem emerged above the water surface, indicating that meristem temperature regulates the growth rate, which fits with our findings. We found reduced growth rate as much as 100 % under LRT (figure 15). Since crop growth is regulated by various processes like canopy radiation capture, photosynthesis and conversion of photosynthate to biomass (Shimono et al., 2002), the reduced growth rate in our case is mainly result of delayed LAR and thus less canopy radiation capture and photosynthesis. Hence, it could be argued that delayed growth rate is attributed by source-limitations.

Leaf growth is influenced more by water temperature than by air temperature (Nishiyama 1976). Although all the plants were grown under the same air temperature, LER was significantly lower under low meristem temperature (as meristem was inside the nutrient solution) (figure 15), indicating that meristem temperature regulates the LER. This is supported by the work of Stuerz (2014), who found decreased LER under lower meristem temperature during the night. Since cell expansion of leaves depends on resources such as carbohydrates, nutrients and light (McMaster et al., 2003), delayed LER could be reason of limited photosynthesis (i.e. lower leaf number). Although a rise in temperature up to 30°C stimulates the growth (Cutler et al., 1980), in our case AGR and LER was lower under high temperature conditions, probably due to a low height of the chamber.

IR64 showed significantly higher AGR and LER under high VPD and LRT conditions whereas Chomrong displayed no changes. Which indicates that high VPD leading accimilation to LRT (Kuwagata et al., 2012; Ahamed et al., 2012) is more favorable for cold sensitive variety (IR64) than cold tolerant variety. IR64 displayed Higher AGR and LER under high VPD and high temperature conditions which can be linked with their origin from tropics.

Under high VPD, IR64 showed no significant difference in AGR and LER between the temperature levels, which is explained by different development stages. Since at this sampling date plants under medium and high temperature were near to panicle initiation stage whereas plants under low temperature were still in early vegetative stage, further growth of the plants under high and medium temperature were inhibited by height of the chamber whereas plants under low temperature remained unaffected.

7.5 Effects on Biomass allocation

LMF was more than 90% lower under LRT compared to the medium and high temperature levels. When meristems were lifted above the water surface, Nagasuga et al. (2011) found 93% decreased dry matter allocation to the leaves under LRT (14^{0}C) compare to the control (25^{0}C), which is linked with decreased single leaf area caused by reduced water potentials of the stem and the growing part of the leaf. In our study, meristems were inside the nutrient solution, therefore decreased LMF is associated with delayed leaf appearance rate leading to lower LN and leaf dry weight.

RMF was more than 80% higher under LRT, which is in contrast with Kuwagata et al. (2012) findings, who found 80% decreased dry matter allocation to the roots under LRT (13^{0}C) compared to the high root temperature (25^{0}C). This difference is related with difference in the temperature treatment. Although plants were 54 days old in their experiment, the LRT treatment started 15 days before the sampling. Therefore plants were under the same development stage. However, in our case, plants were treated from the beginning of the experiment, resulting in slow growth rate, which in turn resulted in plants that were in early vegetative stage under LRT. Therefore, high RMF under LRT is probably result of low total dry weight (TDW) compared to the other two temperatures. Plants allocate more biomass to roots, if the required growth factor is from the root (e.g. Nutrients, water) and more for shoots, if the required growth factor is from the shoot (e.g. Light, CO_2) and this functional aspect might be to increase the plant growth by acquiring most limiting factors (Poorter et al., 2011).However, the underlying mechanism of root chilling induced carbon flux from shoot to root remains unclear and needs further investigation.

Differences in LMF and RMF can be seen between the varieties (figure 15). Under LRT Chomrong showed relatively high LMF and low RMF compared to IR64. Chomrong is a cold tolerant variety (Shrestha et al., 2011) and IR64 is a cold sensitive variety (Dingkuhn et al., 1995). Therefore it could be argued that cold tolerant varieties accumulate more biomass to leaves and cold sensitive varieties more to the roots.

Biomass accumulation between the varieties can also be explained based on the differences in morphology. With small differences in LN between the varieties, higher LMF in Chomrong is due to its larger leaves.

SMF was significantly lower under LRT which is result of low TN. However, IR64 under high VPD showed no changes on SMF between the temperatures due to high TN. Although chomrong displayed less TN, SMF is similar to IR64 in most cases which is explained by thick and long stem of Chomrong. Tall plants are more susceptible to lodging. Since Chomrong was much taller than IR64, Chomrong might be adapted to lodging due to its thick stem.

8. Conclusions and outlook

This work was done to analyze the effects of LRT and VPD conditions on leaf appearance, morphological responses and growth rates. Three different temperatures, two VPD levels and two varieties were used.

All plants were grown under the same air temperature. Hence, delayed LAR and phyllochron under LRT treatment (the effect was more in Chomrong than in IR64) is caused by temperature at meristem which remained inside the nutrient solution. LAR and phyllochron were not affected by VPD treatments.

The physiological and morphological responses appeared to be retarded and so did AGR and LER under LRT, which are linked to delayed LAR. Thus, this experiment successfully proved that growth inhibition of irrigated rice under LRT is the result of source limitations.

Almost all of the observations were significantly higher under medium and high root temperatures. Optimum root temperature of irrigated rice can therefore be estimated between 23.2 ^{0}C and 27.7 ^{0}C. Under these temperatures, IR64 performed well under high VPD whereas Chomrong under low VPD in most cases.

Biomass allocation to stem and leaf decreased whereas increased in root under LRT which remained unaffected by VPD treatments. Differences between the varieties were seen in biomass allocation. Chomrong showed high LMF and low RMF compared to IR64.

An integrated effect of LRT and VPD can be seen in plant parameters, AGR and LER which were higher under high VPD and LRT conditions in most cases. IR64 performed relatively well under these conditions compared to the Chomrong.

By using the information and knowledge obtained by this thesis,

1. It would be useful to conduct further research focused on meristem level to develop cold resistant breeds.

2. It would be worthwhile to analyse root chilling induced carbon flux from shoot to root under different VPD levels and LRT conditions.

3. Further investigation for aquaporin activities under high VPD and LRT conditions would be helpful to achieve a better understanding of the root hydraulic conductivity.

4. The height of the boxes and chambers should be considered prior to conducting this type of chamber experiment to observe significant treatment effects.

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