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Physiological aspects of water use efficiency  
in selected irrigated rice genotypes grown in the Sahel

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## **Abstract**

With a growing world population, water demand in agriculture is rising, while water available for irrigation is becoming scarcer due to limited resources and additionally increasing water consumption like industrial and municipal use. This scarcity contrasts with the rising rice demand of a growing world population. Rice depends on high irrigation amounts to produce high yields. It is important to find ways leading to higher water use efficiency in rice production. One way is to apply water-saving irrigation.

Observations on important physiological parameters are often labour-intensive and/or not very precise. For precise leaf area measurements, single leaves of the entire hill need to be scanned. Transpiration can be roughly estimated on field level by subtraction of evapotranspiration and evaporation, which can be determined with lysimeter measurements. For reliable determination of those important parameters, better and less complex methods are needed.

A field experiment was conducted at the AfricaRice Sahel Station, Senegal to validate a method of leaf area determination via specific leaf area (SLA), and to estimate transpiration over the cropping period via single leaf transpiration measurements. Furthermore, leaf development and phyllochron were observed. All parameters were observed on lowland rice under flooded and water-saving conditions. In the flooded treatment, a constant water layer of about 10 cm was maintained over the season. In the water-saving treatment, the soil was kept at saturation level without standing water to avoid evaporative losses. To assess the influence of different seasons on these parameters, two sowing dates were included, one in the hot wet season and one in the cold dry season.

Samples for SLA were taken 3 to 5 times during the growing periods, separated by leaf levels, scanned and dry weight was determined. Transpiration rate was measured on 3 replications on all active leaves of the main culm twice a week at noon and weekly up to 5 times over the whole day. Leaf area was calculated for SLA of different leaf level combinations and compared to leaf area calculation with the last leaf of the main tiller and the whole leaf area. With transpiration rates, combined with leaf area and weather data, total transpiration of the measuring period is estimated and compared with calculated water loss. Differences between seasons, treatments and varieties were reviewed.

Water-saving irrigation and also a colder environment decrease both leaf area and transpiration rates of rice hills. The best solution for estimation of leaf area is a combination of leaf level of the youngest fully developed leaf and the following older one and inclusion of 3 to 5 tillers

additionally to the main tiller to cover tiller effects on leaf area. Estimated and calculated transpiration showed minor differences only.

Keywords:

Lowland rice, phyllochron, SLA, transpiration, water-saving irrigation

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## 1 Introduction

Rice is one of the most important crops worldwide; together with wheat and corn it provides 60% of human food (Tilman et al. 2002). World rice production in 2008 amounted to 685 million tonnes (FAO 2008). In Senegal, rice is one of the most important cereals. Estimated per capita rice consumption ranges from 70 to 75 kg per year (USDA Foreign Agricultural Service 2007). In 2008, 368 thousand tonnes were produced in Senegal, which is 0.05 % of worlds total rice production (FAO 2008). Local rice production meets only about 20 % of the country's needs. To meet the consumption needs of the population, Senegal imports rice. In fact, it is the second largest rice importer in Africa. (USDA Foreign Agricultural Service 2007).

Irrigation water for rice production in the north of Senegal is derived from the Senegal River. Competition among user groups for water from the Senegal river will become more severe in the future, as demands for domestic use from the Dakar metropolitan area will increase, whereas water availability is expected to decrease (de Vries et al. 2010).

With increasing water scarcity and the need to feed the growing world population, it will become more and more important to save water. It is particularly important to save water in agriculture, because agriculture is the largest consumer of water (using 72 % of total water worldwide), while the demand of water for industrial, municipal and other uses increases and less water will be available for agriculture as the opportunities for developing new water resources for irrigation are limited (Barker et al. 1998). An option to achieve food security and alleviate poverty other than expansion of cropped area which causes environmental degradation, destruction of natural ecosystems, and loss of biodiversity, is to increase yields and productivity on existing crop land (Tilman et al. 2002). To increase yield and total production with current management practices, more water will be needed to meet the increased transpiration requirements, which may not be possible due to water shortage, so it is important to increase the water productivity of rice (Bouman et al. 2007). To increase the water productivity, major changes in practices, policies, and institutions will be required to ensure limited water resources being appropriately managed (Barker et al. 1998). If these steps are not taken, rice will be the crop most affected, as it depends most heavily on irrigation and is extremely sensitive to water shortage (Bouman et al. 2001).

Many scientists work on water productivity and water-saving in rice and many different solutions are recommended to increase the productivity of irrigation water, for example by increasing the value of output per unit of water transpired, reducing losses to

evaporation, seepage and percolation (by applying saturated soil culture or alternate wetting and drying regimes) or reusing water (Barker et al. 1998).

This work was conducted in the framework of the RISOCAS project (Developing rice and sorghum crop adaptation strategies for climate change in vulnerable environments in Africa). The RISOCAS project in Senegal focuses on genotypic physiological adaptation mechanisms of irrigated rice for specifically targeted environments in the context of climate change. 10 strongly contrasting genotypes are grown in staggered sowing dates to study varietal responses to different environments in terms of phenology, yield and water use.

The present work focuses on transpiration and specific leaf area. Transpiration on plot level can be estimated (like in the ongoing RISOCAS trials) via subtraction of results gained with lysimeters for evapotranspiration and evaporation. This method is time consuming and labor intensive, because measurements have to be taken after irrigations (minimum twice a week) and also cost intensive, because metal for lysimeters is expensive but susceptible for corrosion. Another method of measuring transpiration was investigated. In order to calculate leaf area index (LAI), specific leaf area (SLA) is observed in the RISOCAS trial on youngest fully developed leaf of the main tiller. The focus on SLA was laid to investigate in differences when using other leaves or combinations. Observations on phyllochron were made to compare different genotypes at the same physiological age.

## 2 Hypotheses & Objectives

Following hypotheses were developed:

1. Phyllochron is delayed under water-saving irrigation and also in the cold season
2. All genotypes develop a larger leaf area and greater specific leaf area in the hot season, both are reduced under water-saving irrigation.
3. It is possible to estimate total leaf area accurately at any time using the SLA of the youngest fully developed leaf.
4. Transpiration strongly depends on leaf level, micro-climatic conditions, water-supply and genotype.

Objectives:

1. Assessing effects of season and water-saving irrigation on phyllochron, leaf area, specific leaf area, and transpiration.
2. Finding the best combination of SLA of leaf levels to estimate leaf area as accurately as possible while trying to minimize effort.
3. Estimating transpirational water-loss on plot level via gas-exchange measurements on individual leaves.

### 3 Literature review

#### 3.1 Water use and water-saving rice production

Rice is one of the most important crops worldwide; it is the staple food for 3 billion people. About 90% of the world's rice production is harvested from irrigated or rainfed lowland rice fields (Bouman et al. 2007). Because rice is extremely sensitive to water shortage (Bouman et al. 2001) it is mostly grown under flooded conditions. Rice production is one of the biggest consumers of the world's freshwater resources. Within agriculture, it accounts for approximately 30 % of the total irrigated area. To produce 1 kg of grain, farmers have to put 2 to 3 times more water in rice fields than in those of other cereals (Barker et al. 1998; Bouman et al. 2007).

In lowland rice production, water is needed for land preparation and to match the outflows by seepage (lateral flow of water through bordering bunds), percolation (vertical flow of water from the ponded water layer to below the root zone), evaporation and transpiration during crop growth (Bouman et al. 2007). The only productive water use of these outflows is transpiration, because it directly linked to assimilation via stomatal conductance. After crop establishment, water is usually kept ponded until shortly before harvest (Bouman et al. 2001).

However, the scarcity of and competition for water have been increasing worldwide (Barker et al. 1998). Haefele et al. (2009) stated that unproductive water losses (evaporation, percolation, seepage, transpiration from weeds) must be reduced, and the quantity and/or the water-use efficiency (defined as total above ground dry mass per unit water applied via irrigation and rainfall (Borrell et al. 1997)) need to be increased. Borrell searched an optimal irrigation method for rice production in semi-arid environments and found, the selection of the method should not be based on the criterion of water-use efficiency alone but rather on the dual criteria of water-use efficiency and total water use.

Possible techniques to save water and increase water productivity (grain yield per volume of water used (Won et al. 2005)) without decreasing land productivity are saturated soil culture (SSC), alternate wetting and drying (AWD), aerobic rice (AR), and drip and sprinkler irrigation (Tuong et al. 2005).

Bouman et al. (2001) stated that the most promising option to save water and increase productivity is to reduce the ponded water depth from 5 -10 cm to the level of soil saturation, which is the technique of SSC. They showed that it is possible to save 23 %

water with only 6 % yield loss: SSC reduced the amount of water use (WU) (water applied via irrigation and rainfall) through reduction in losses due to deep percolation and evaporation (Tuong et al. 2005) while maintaining an adequate water supply to the rice plants - even when total WU decreased by 25 % - with only small effects on physiological and morphological characteristics (Nguyen et al. 2009). Borrell et al. (1997) reported substantial reductions in variable costs of production (e.g. pumping water) attainable with SSC by reducing water use without or with small reduction of yield and quality.

One of the main problems of water-saving irrigation techniques, however, is the risk of yield reduction caused by potential drought-stress effects on the crop (Bouman et al. 2001). Moreover, implementing SSC requires good water control at the field level and labour-intensive frequent irrigations. Another major problem of water-saving irrigation is weed infestation, which may cause a complete yield loss in direct seeded rice in absence of weed control (Tuong et al. 2005).

### **3.2 Phyllochron and leaf development of rice**

Counting the leaves on the main culm is the best way to provide a physiologically meaningful age for rice plants (Yoshida 1981). The first leaf appears to lack a blade, from the second leaf, all leaves develop a leaf blade and from the third leaf, leaves emerge after each preceding leaf has fully elongated. The appearance of consecutive following leaves can be observed in two different ways: regarding the leaf tips exceeding the leaf sheath or the appearance of the ligule to consider a leaf as fully developed. However, nearly perfect agreement for appearance of a leaf tip and its preceding ligule was found and so appearance of leaf tip was suggested to be at the same day as the preceding leaf ligule (Yin et al. 1996).

Most early- to medium- maturing varieties develop about 10 to 18 leaves on the main culm. In photoperiod-insensitive varieties, the number of leaves is constant under most conditions. At a given time, the rice plant is composed of leaves that are physiologically different in age and activity. Before the initiation of panicle primordia, a leaf emerges every 4 to 5 days, afterwards every 7 to 8 days. The life span of individual leaves after elongation differs widely among leaves. Upper leaves have longer life spans than the lower ones (Yoshida 1981).

To compare and give an overview about leaf parameters, phyllochron development is used. Yin et al. (1996) defined phyllochron as the interval between the appearance of two successive leaves during the development of rice leaves. The maximum value of

phyllochron can always be found around the time of floral initiation (Itoh et al. 2006). Phyllochron of tillers is closely linked to the development of phyllochron on the main culm (Jaffuel et al. 2005).

Phyllochron is mainly affected by temperature, day length and genotype (Itoh et al. 2006; Sié et al. 1998b). Sié et al. (1998a) postulated that the vegetative growth stages depend on temperature and later reproductive stages depend on the photoperiod. And he also found that temperature mainly affects duration, because e.g. low temperatures increase crop duration mainly through delayed leaf appearance. Yin et al. (1996) observed an increasing leaf number with extended crop duration due to photoperiod. All these authors only considered air temperature to influence phyllochron in rice. Yin et al. (1996) showed in detail, that leaf appearance is controlled by temperature near the apical meristem and the importance of considering water temperature when working with field grown rice in regard to phyllochron.

### **3.3 Leaf area and specific leaf area of rice**

Specific leaf area (SLA) is a measure of leaf thickness (Yoshida 1981). It is calculated as leaf area (LA) in m<sup>2</sup> over leaf dry weight in kg. SLA varies strongly among individual leaves and leaf growth stages (Tardieu et al. 1999).

SLA is not a constant parameter, but depends on the crop developmental stage. During development of a rice plant, it starts relatively high and decreases exponentially over time to a minimum value at flowering. SLA is affected by growing conditions e.g. temperature, genotype, and development stage whereas it is not so much affected by variability of leaf and tiller size caused by resources (Asch et al. 1999; Dingkuhn et al. 2001). However, Tardieu et al. (1999) reported, that large variations in SLA are caused by changes in leaf expansion rate and photosynthetic rate depending on plant water status and time of day. They also found a 30 % decrease in SLA between morning and afternoon in all leaf zones of sunflowers and thus recommend taking the time at which leaves are collected and the environmental conditions into account before interpreting the data.

Specific leaf area influences cultivar differences in vegetative growth vigor, light interception, potential growth and tillering ability. (Dingkuhn et al. 1998; Asch et al. 1999). For example Asch et al. (1999) reported that a great SLA is linked to early growth vigor. Furthermore SLA is likely to be higher in long- than short-duration cultivars and is generally greatest in *O. glaberrima* and *O. sativa indica* groups (Dingkuhn et al. 1999).

SLA determines the cost of producing leaf area; a small SLA (which correlates with thick leaves) implies more assimilates were needed to produce a given LA (Dingkuhn et al. 2001). The cultivar with the largest SLA also developed the largest LA (Asch et al. 1999). Leaf area increases with time, depending on environmental conditions such as water deficit, temperature and evaporative demand. Tardieu et al. (1999) modeled leaf area as the product of epidermal cell number and mean area of epidermal cells. Dingkuhn et al. (2001) found that leaf area is positive correlated with SLA and tillering ability and SLA has a much greater effect on leaf area than assimilate partitioning ratios, light extinction coefficients, and mean leaf tip angle.

Leaf area index (LAI) is defined as the ratio between leaf area and unit of land area. LAI has an influence on light interception, crop growth, crop water use, and crop-weed competition (Sone et al. 2009). Dingkuhn et al. (1990) showed that there are differences between direct seeded and transplanted rice in the development stage where maximum leaf area index is reached: maximum LAI coincided with heading in transplanted rice but occurred shortly after panicle initiation in direct-seeded IR64 because transplanting shock delayed development.

In rice, it is common to measure only the area of leaf blades to determine leaf area, because photosynthesis by sheaths and culms is negligible (Yoshida 1981). Leaf area can be measured with different methods, different measurement systems, such as described by Sone et al. (2009) or destructive methods. Destructively monitoring leaf area during the cropping season requires labor-intensive measurement of the total leaf area over a specific area of land surface, involving removal of a number of plants at the soil surface, separation of leaves from the other plant parts, and determination of their area with a leaf area meter (Sone et al. 2009).

### **3.4 Transpiration of rice leaves**

Transpiration is the loss of water in the form of vapor from plant surfaces. It occurs mainly through stomata and to a much smaller extent through the cuticle. Transpiration is controlled primarily by opening and closing of stomata. It is not possible to avoid transpiration which can lead to water deficits in plants. Transpiration losses increase with leaf area and reach a plateau at a leaf area index of 3.5 to 4. At this plateau, transpiration loss on sunny days accounts for about 90 % of evapotranspiration losses (Yoshida 1981).

The gas exchange of a rice canopy is affected by SLA and leaf area, because e.g. a high SLA reduces the amount of assimilates needed to produce a given leaf area,

resulting in earlier ground cover, and therefore, a greater light harvest and higher canopy photosynthesis rates early in the season (Dingkuhn et al. 1999). Yoshida (1981) also linked canopy photosynthesis to leaf area, he explained that gross photosynthesis of a canopy increases curvilinearly with increasing leaf area, because as leaf area increases, lower leaves become shaded and mean photosynthetic rate of all leaves decreases.

Transpiration of single rice leaves differs according to their position on the culm (Yoshida 1981). The first two leaves from the top had the highest photosynthesis while the fourth leaf on the main culm (counted from the top) had a very low net photosynthetic rate (Yoshida 1981), caused by shading and progressing leaf age. The rate of photosynthesis and transpiration for single leaves is not stable during development of plants and leaves. Transpiration of single leaves starts high and decreases during time, like Fukai et al. (1985) for example showed with the rate of photosynthesis of flag leaves that decreased during grain filling in all cultivars. Furthermore transpiration influences the direct environment of rice leaves, for example O'Toole et al. (1982) showed that increased transpiration decreased leaf temperature and leaf water potential in rice.

Transpiration rate is also affected by water deficits (Fukai et al. 1985). Wopereis et al. (1996) found that drought affected transpiration rates by closure of stomata and changes in leaf morphology (relatively abrupt decline in leaf expansion) of the rice plant. Contrasting, Turner et al. (1986) showed that there is no adaptation to less water availability regarding gas exchange of dryland rice varieties, because no difference was found between dryland and wetland cultivars in leaf photosynthesis. Tanguilig et al. (1987) reported that rice is not able to control transpiration in the same extent as maize and soybean. He compared transpiration of rice, maize and soybean while they were subjected to water stress. At the same leaf water potential, rice transpired more than maize and soybean, so it was not possible for rice to survive as long as maize and soybean under water stress.

Transpiration changes during the course of a day in response to meteorological variables like air temperature, vapour pressure deficit (VPD) and solar radiation (O'Toole et al. 1982), while the degree of the afternoon depression depends on plant water deficits and is induced by partial stomatal closure (Dingkuhn et al. 1990). Solar radiation and wind speed both show strong interactions with VPD in their effects on diurnal changes in transpiration rate. The diurnal trend of transpiration appears to be more directly influenced by VPD and wind speed than solar radiation (O'Toole et al. 1982; Dingkuhn et al. 1990).

Midday depression in transpiration and photosynthetic rate is mentioned by several authors. Turner et al. (1986) reported that the rate of net photosynthesis decreased after 9.00 h and increased again after 14.00 h, while O'Toole et al. (1982) described transpiration rate to begin increasing at sunrise and attained the maximum value during the period 12.00 -15.00 h.

Transpiration can be measured through a photosynthesis measurement system or estimated through subtraction of evapotranspiration and evaporation, which can be measured directly using lysimeters or indirectly from changes in soil water (Farahani et al. 2007).

## 4 Material and Methods

### 4.1 Experimental site and growth conditions

The experiments were conducted at the Sahel station of AfricaRice in Senegal, West Africa. The station is located in Ndiaye (16°14' N 16°14' W), 35 km northeast of Saint Louis at the northern border of Senegal to Mauritania in the Senegal river delta.

The region of the Senegal River valley and delta is regarded as representative for irrigated rice cropping in the Sahel.

Senegal is located within the transition zone between Sahel and humid tropics. It is characterized by two main seasons: a wet season from July to October and a longer dry season from November to June which can be divided into cold dry and hot dry season.

Asch et al. (2001) and Dingkuhn et al. (1995) reported, that the wet season is characterized by about 200 mm of rainfall and daytime air temperature between 22 and 40 °C whereas the hot season is characterized by almost no precipitation and daytime air temperatures between 15 and 45 °C in the hot dry season and 12 to 38 °C in the cold dry season.

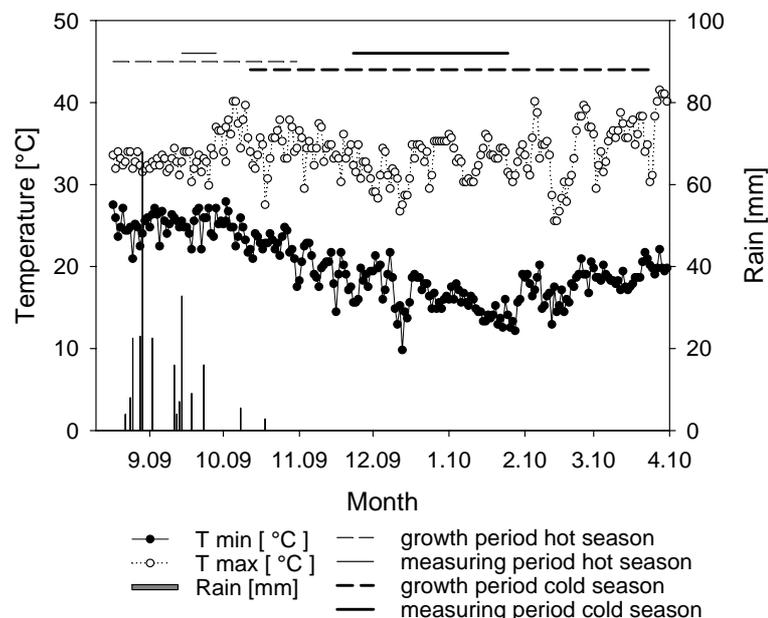


Figure 1 Recorded data of temperature [°C] and precipitation [mm], taken by the AfricaRice field micrometeorological station. Thin lines at the top represent growth (solid line) and measuring (dashed line) period in the hot wet season and thick lines represent the periods in cold dry season respectively.

The presented study included two sowing dates of the RISOCAS trial: mid of August and October 2009. Rice sown in August grew in the hot wet season and October sown rice in the cold dry season.

Daily temperature and precipitation of the second half of 2009 until March 2010 are presented in Figure 1. The data shown include the two growing cycles.

Precipitation in the hot wet season was 241.1 mm, whereas there was no rain during the growing period starting in October. Mean air temperatures were 28.2 °C in hot wet season and 24.7 °C in cold dry season. Until end of September, the maximum air temperature was 33 °C (average), afterwards it increased to an average of 36 °C in the first week of October and declined slightly.

The soil was a heavy, slightly acid (pH 5±6) vertisol clay with 47 % clay, 40 % silt and 13 % sand (Asch et al. 2001).

## **4.2 Experimental design, genotypes, and water treatments**

### **Varieties**

Measurements were conducted in the RISOCAS trial. 5 rice varieties were chosen for observations in this study. Chosen varieties were known from the RISOCAS trial to react differently to different water treatments and seasons. These varieties are listed in Table 1.

*Table 1 Chosen varieties for measurements, sat=sativa, jap=japonica, ind=indica, Phy=phyllochron, E=Transpiration. Source: Schlegel 2009 (modified).*

<b>Varieties</b>	<b>Species / Subspecies</b>	<b>Type</b>	<b>Duration</b>	<b>Country of Origin</b>	<b>Special Property</b>	<b>Measurements</b>
<b>CG14</b>	glaberima	traditional	short	Senegal	upland	SLA
<b>Chomrong</b>	sat/jap	traditional	short	Nepal	cold tolerant	SLA
<b>IR4630-22-2</b>	sat/ind	improved	medium	Phillipines	salt tolerant	Phy / SLA / E
<b>IR64</b>	sat/ind	improved	short	Phillipines	intern.check	Phy / SLA / E
<b>Sahel202</b>	sat/ind	improved	medium	Nigeria		SLA

Three varieties were only used for observations on SLA (Chomrong, CG14, and Sahel202) whereas IR4630-22-2 (further abbreviated as IR4630) and IR64 were used also for phyllochron and transpiration measurements.

## Experimental design

Each sowing date consisted of two blocks with different irrigation treatments. 10 varieties (from which 5 were used for this work) in three replications are grown in each block. Each block was divided in 30 plots of 3.2 m x 4.2 m with a spacing of 20 cm (300 rice plants per plot). Plots were separated by bunds.

Each plot was divided into different areas, which were used for yield determination (84 hills in the centre of the plot) or different types of samplings. A typical plot design is shown in Figure 2. Plants for phyllochron and transpiration measurements were chosen in the third row from the bunds to minimize boundary effects but allow practical transpiration measurements. Samples for SLA were always taken at the same day as the bordering samples of the RISOCAS trial.

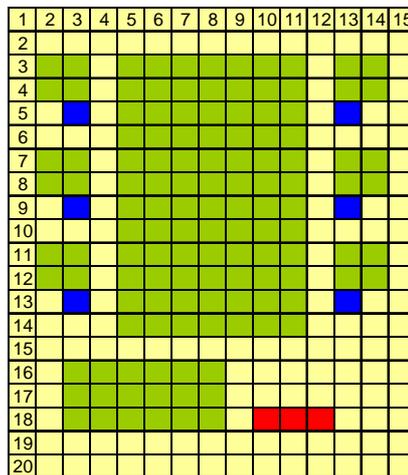


Figure 2 Plot design, green areas: sampling and harvest area of the RISOCAS trial, red areas: Phyllochron and Transpiration measurements, blue areas: SLA samplings. Source: S. Stürz, modified.

## Water treatments

From onset of tillering, two different water treatments were applied. These treatments were separated in two blocks with 10 m distance in between to avoid water flow. First treatment, from now called “flooded treatment”, was the conventional method of growing irrigated rice, having a constant standing water layer of minimum 5 cm in the plots. In the second treatment, called “water-saving treatment”, where the Saturated Soil Culture system was used, the soil was kept in a saturated state without standing water.

## **Sowing dates**

Observations and measurements were taken in two seasons in order to compare the responses of the rice plants with regard to different growing conditions. These seasons were represented by two sowing dates of the RISOCAS trial: 17th of August (which is situated in the hot wet season) and 12th of October 2009 (cold dry season).

## **4.3 Samplings, measurements, and calculations**

### **4.3.1 Measurements in the RISOCAS trial**

#### **Weather**

Weather data were continuously taken by the micro-meteorological station of AfricaRice near the field. Temperature, relative humidity, wind speed, rain and solar radiation were automatically recorded. Minimum temperature, relative humidity, maximum wind speed, and solar radiation were used in a cluster analysis to group similar days for the calculation of transpiration over the cropping period.

#### **SLA to calculate leaf area**

For specific leaf area, the four youngest fully developed leaves of the main tiller of individual hills were taken from each plot, combined, and scanned for leaf area. Afterwards leaves were dried at 70 °C until constant weight and weighted. SLA was calculated as leaf area in m<sup>2</sup> divided by dry weight in kg. Hills were sampled, separated in stems and leaves, dried and weighted. For leaf area estimation, SLA of the youngest fully developed leaves was multiplied with the dry weight of the leaves of individual hills.

#### **Phenology**

Phenological stages were observed for all varieties of the RISOCAS trial. Observed stages included beginning of tillering, panicle initiation (PI), flowering, and maturity.

### **4.3.2 Phyllochron and nomenclature of leaves**

Phyllochron was observed for 3 hills per plot for IR4630 and IR64 in both seasons and treatments. Starting from appearance of the first leaf, plants were observed for new fully developed leaves on the main culm three times per week. A leaf was defined as “fully developed” when the tip of the next leaf was visible and the ligule was found (which was usually the same day). Leaves of the main culm were marked with a pen, the first leaf that appeared (which lacks a leaf blade) was counted as “1”, up to the flag

leaf which had the highest number. Dates of completed development and leaf death, defined as 50 % dead leaf area, were noted and averaged for the three hills per plot.

### 4.3.3 Leaf area and specific leaf area

Samples for SLA of IR4630, IR64, Chomrong, CG14, and Sahel202 were taken 3 times in the hot wet season (at 43, 57, and 71 days after sowing (DAS)) and 4 to 5 times (29, 44, 65, 88, and (105) DAS) in the cold dry season depending on crop duration of individual varieties. SLA samples were always taken between 8 and 9 am so that differences in SLA caused by time of the day could be excluded (Tardieu et al. 1999). In order to compare (specific) leaf area development for varieties with different durations until maturity, development stages were standardized. For this, days from sowing to flowering were plotted as quotient of 1, days from flowering to maturity as quotient of 1 plus 1, resulting in numbers from 1 to 2. Development stages were used to compare varieties with different durations at the same development stages. Phenology data of the RISOCAS trial were taken to define date of sowing as 0 DVS and flowering as 1.0 DVS. Development stages of SLA samplings for the five varieties are presented in Table 2. Missing values at flowering were due to rat damage in the water-saving treatment.

*Table 2 SLA samplings in days after sowing (DAS) for the hot wet and the cold dry season and the respective development stages (DVS) for the five varieties in both treatments (F=flooded and WS= water-saving treatment), cursive numbers show missing data of flowering (flowering dates of flooded treatment were taken).*

		Development Stage (DVS)									
		IR4630		IR64		Chomrong		CG14		Sahel202	
Season	DAS	F	WS	F	WS	F	WS	F	WS	F	WS
hot wet	<b>43</b>	0.53	0.52	0.72	0.70	0.85	0.82	0.70	0.66	0.58	0.51
	<b>57</b>	0.70	0.70	0.96	0.93	1.13	1.08	0.92	0.87	0.76	0.68
	<b>71</b>	0.88	0.87	1.20	1.16	1.42	1.35	1.16	1.09	0.96	0.85
cold dry	<b>29</b>	0.21	<i>0.21</i>	0.32	<i>0.32</i>	0.32	<i>0.32</i>	0.27	<i>0.27</i>	0.28	<i>0.28</i>
	<b>44</b>	0.33	<i>0.33</i>	0.48	<i>0.48</i>	0.49	<i>0.49</i>	0.40	<i>0.40</i>	0.43	<i>0.43</i>
	<b>65</b>	0.48	<i>0.48</i>	0.71	<i>0.71</i>	0.73	<i>0.73</i>	0.60	<i>0.60</i>	0.64	<i>0.64</i>
	<b>88</b>	0.65	<i>0.65</i>	0.96	<i>0.96</i>	0.99	<i>0.99</i>	0.81	<i>0.81</i>	0.86	<i>0.86</i>
	<b>105</b>	0.78		1.14		1.18		0.96		1.03	

At SLA samplings, one plant of each replication, variety and treatment was sampled and leaves were separated regarding their position. Leaves of every culm were

counted from the top to define leaf levels. The youngest fully developed leaves were called “leaf level 1 (L1)”, the consecutive leaves to the bottom were leaf level L2 to L4. Not yet fully developed leaves belonged to the leaf level L0. Leaves of one leaf level were combined and scanned to determine leaf area of the respective leaf level. Leaf area of the whole hill was calculated as sum of leaf area L0 to L4. Scanned leaf area of the hill was called “measured leaf area”. Leaves were oven dried at 70 °C for at least one week and weighted to measure dry weight of leaf levels. SLA of hills or leaf levels was calculated as leaf area divided by leaf weight.

For SLA of leaf levels, all leaf level combinations were calculated; L1+2 equals the combination of leaf level 1 and 2 and so on. With SLA of leaf level combinations, leaf area of the combinations was calculated by weight of the whole hill multiplied by SLA of the leaf level. Leaf areas of leaf levels were compared with the measured leaf area. This method of leaf area calculation was used to validate leaf area calculation of the RISOCAS trial.

Curves for leaf area development within the growing period were interpolated for daily LA with equation 1:

$$\ln y = a + bx + cx^{(0.5)} \quad \text{(Equation 1)}$$

#### **4.3.4 Transpiration**

Transpiration was measured with a LCi, a portable photosynthesis measurement system from ADC BioScientific Ltd. (Great Amwell, England) which is especially designed for field measurements. Measurements started 28 DAS (hot wet season) and 42 DAS (cold dry season).

Leaves 9 to 13 of the main culm for one hill of each replication and treatment of IR4630 and IR64 were measured two times a week between 11.30 am and 1.30 pm (time of maximum solar radiation) to determine development of transpiration over the growing period. Measurements were corrected for leaves that were thinner than the measuring chamber (1 cm) by multiplying the transpiration rate with the width of the leaves. Floating averages were calculated as average of always three following values.

Diurnal development of transpiration was measured once a week at the same plants and leaves. Each plant was measured at least 4 times with interval of more than one hour and time of measurement was noted. Plants for measurements were changed when a leaf was torn to avoid measuring errors because of discontinuous xylem flow.

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For estimations of genotypic and seasonal transpirational losses, continuous transpiration rates over the day were calculated from measured points for leaf level 1-4 of the flooded and the water-saving treatment of IR4630 and IR64 via interpolation with a Gaussian curve (Equation 2) using TableCurve 4.0 (Systat Inc., 2001).

$$y = a \exp(-0.5((x-b)/c)^2) \quad \text{(Equation 2)}$$

#### **4.4 Data analysis**

Data analysis for transpiration measurements was done with SAS (Proc glm) to test for differences between measurement days, treatments, varieties, and leaf levels.

Students T-test in Excel was used to compare leaf area between the sowing dates and between this work and the RISOCAS trial.

## 5 Results

### 5.1 Phyllochron and leaf development

Phyllochron and leaf development are shown in Figure 3 and 4 for 2 irrigation treatments in the hot wet and the cold dry season for IR4630 and IR64, respectively. Seedling emergence was observed at 4 days after sowing (DAS) in the hot wet season and at 10 DAS in the cold dry season.

The total number of leaves that appeared until maturity differed among the varieties and was influenced by irrigation treatment and season. In IR4630 in the hot wet season 14 leaves appeared on the main culm under both irrigation treatments, whereas in IR64 16 leaves appeared on the main culm under conventional irrigation and only 14 under water-saving irrigation. In the cold dry season in IR4630 16 leaves appeared under conventional irrigation as compared to 15 in IR64 whereas under water-saving irrigation 14 leaves appeared in IR4630 compared to 13 in IR64.

Leaf appearance of the first nine leaves was not significantly different between varieties and treatments at the same sowing date. In general, leaves appear 4 (IR64, water-saving treatment) to 32 (IR64, flooded) days earlier in the hot wet season than in the cold dry season. In the hot wet season, phyllochron of IR64 was up to 14 days shorter relative to IR4630. The same leaves of IR4630 appeared in both treatments at the same time whereas in IR64, leaf appearance was delayed by up to 5 days in the water-saving treatment. From leaf 10 in the cold dry season, phyllochron was delayed in the water-saving treatment, e.g. leaves of IR4630 appeared 4 – 18 days later as compared to the conventional irrigation treatment. A similar trend but less pronounced was observed for IR64 and leaf development rate decreased for both varieties in the water-saving irrigation.

Leaves with the same number of appearance displayed a strongly decreased senescence rate in the cold dry season (up to 76 days longer survival of leaves from IR64 under water-saving irrigation) compared to the hot wet season. In the hot wet season significant differences in genotypic responses to the irrigation treatment were observed. Depending on the leaf level, leaf development of IR64 was 1 to 24 days (conventional irrigation) and 1 to 31 days (water-saving treatment) faster than in IR4630. In contrast, in the cold dry season, no genotypic differences were observed while leaf duration differed among treatments between 2 and 31 days.

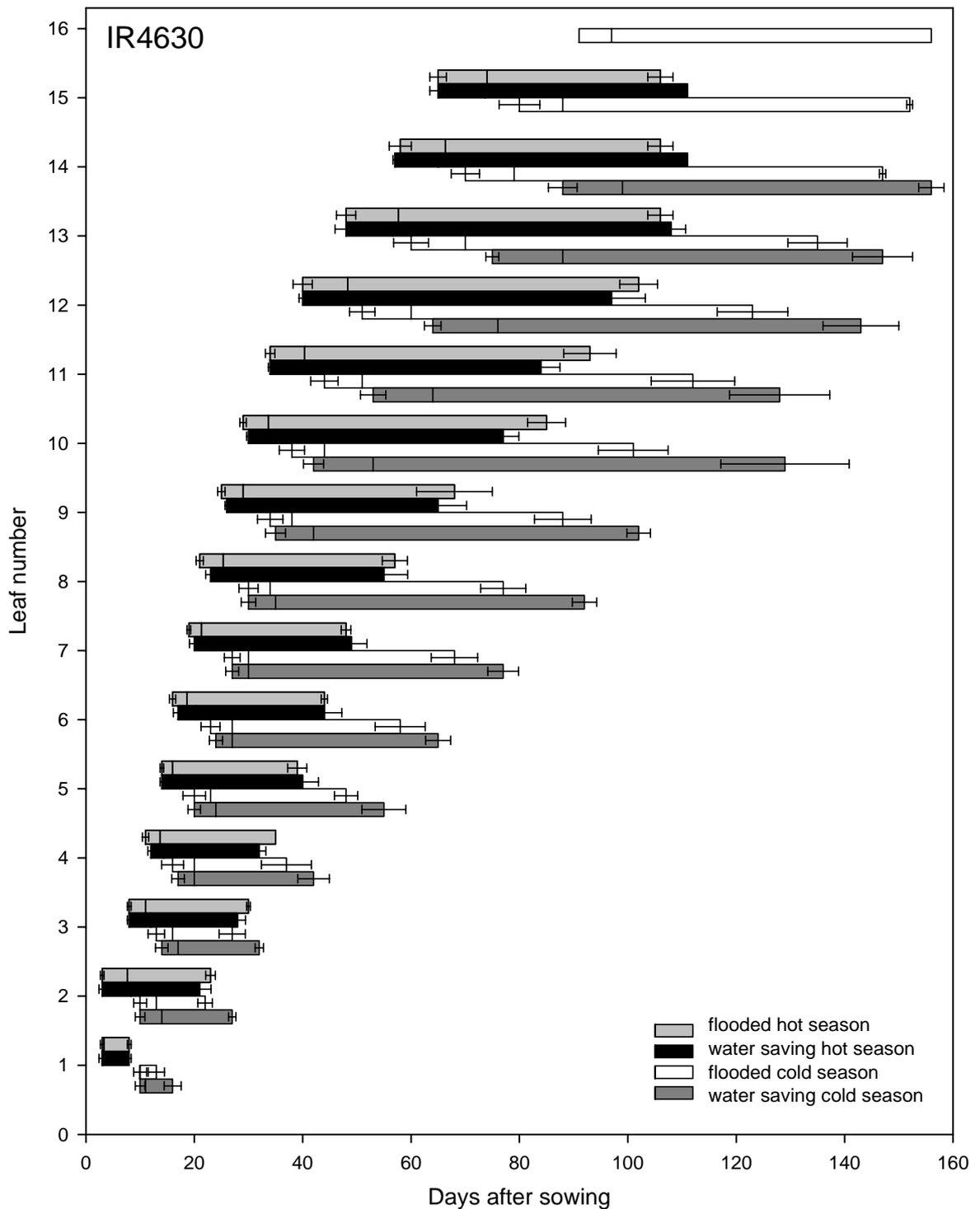


Figure 3 Leaf Development of IR4630 in two seasons, hot wet season (gray and black) and cold dry season (white and dark gray) and two treatments (flooded and water-saving). Leaf appearance = beginning of the bar, point of full elongation = vertical line inside, leaf death = end of the bar, leaf duration = whole length. Error bars show standard errors of means.

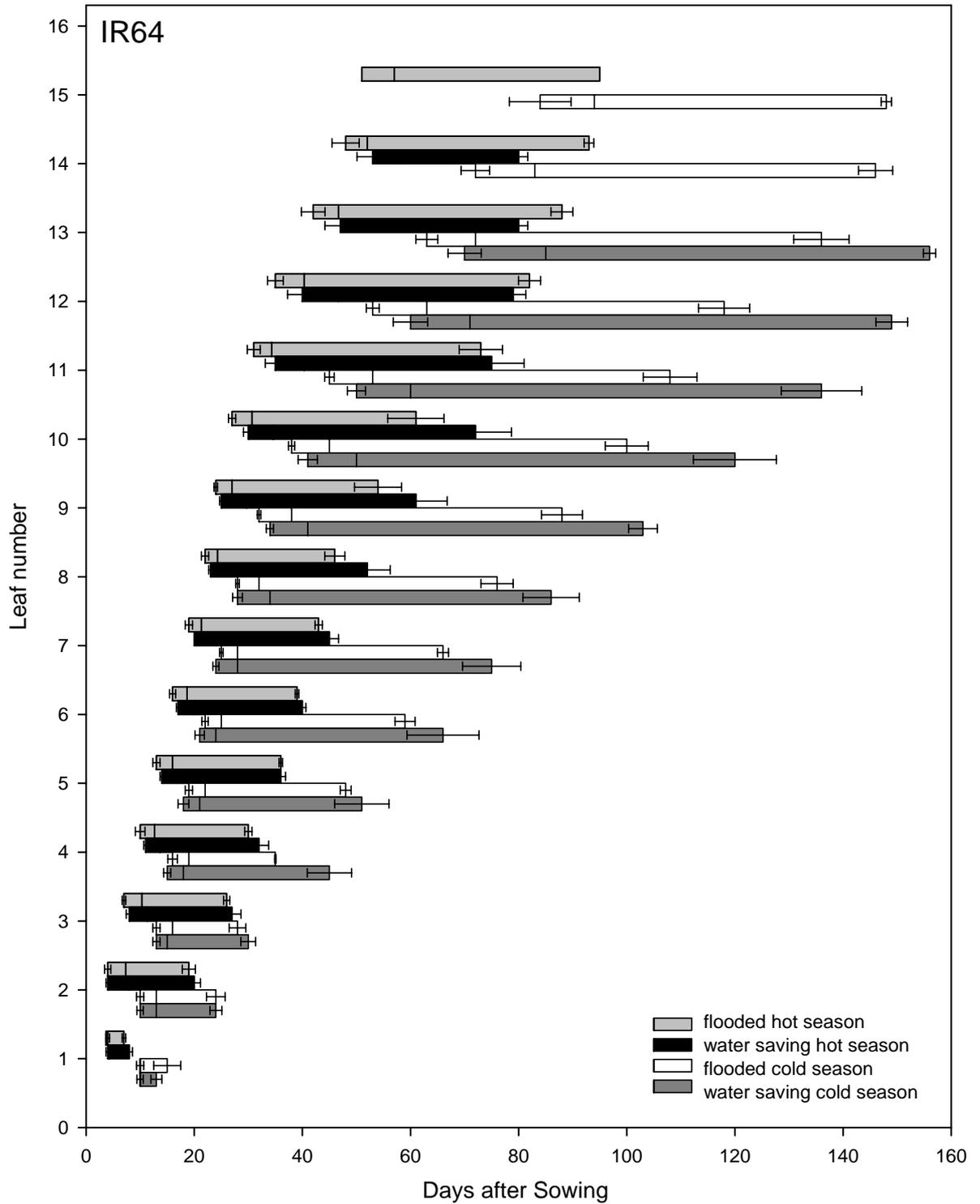


Figure 4 Leaf Development of IR64 in two seasons, hot wet season (gray and black) and cold dry season (white and dark gray) and two treatments (flooded and water-saving). Leaf appearance = beginning of the bar, point of full elongation = vertical line inside, leaf death = end of the bar, leaf duration = whole length. Error bars show standard errors of means.

For leaf 3 to 8 (water-saving treatment) and 9 (control treatment) phyllochron was constant at about 3 days for both genotypes and seasons, for later leaf levels phyllochron increased to 9 days for IR4630 and 6 days for IR64 (hot wet season) and to 16 days and 15 days in the cold dry season. From leaf 9 (10) on, the development was up to 8 days faster in the hot than in the cold season. In the hot wet season phyllochron between leaf 9 and the last leaf to appear was larger in IR4630 than in IR64 with minor differences between treatments.

Total leaf duration varied depending on genotype, treatment and sowing date between 1 to 10 days for the first leaves and up to 24 days for the longest individual leaf duration. In general, cold dry season conditions increased individual leaf duration in both genotypes as compared to the hot wet season and water-saving irrigation enhanced this effect. Genotypic responses to irrigation treatment in the hot wet season were small and not consistent.

## **5.2 Leaf area development**

### **5.2.1 Leaf area development of one hill**

For both irrigation treatments, 5 varieties, and 3 replications 1 hill each was sampled 3 times during the hot wet season and 4-5 times during the cold dry season. Leaves were separated from the culm and scanned according to their levels counted from the top for five leaf levels. Total leaf area per hill was calculated as the sum of the level specific areas. Leaf area development was compared for conventional and water-saving irrigation treatment, sowing date, and varietal differences.

#### ***5.2.1.1 Flooded – water-saving (comparison of treatments)***

Figure 5 shows the development of leaf area per hill for five varieties, 2 treatments, and 2 sowing dates. Leaf area ranged between 0.003 m<sup>2</sup> (averaged) early in the season and 0.23 m<sup>2</sup> at the end of the season. Leaf area development was significantly different between the two planting dates with an on average 1.6 times larger leaf area in the hot wet season than in the cold dry season. Genotypic differences as well as treatment effects were small early in the season but became in most cases more pronounced as the season progressed. Water-saving irrigation resulted in smaller leaf area as compared to the conventional irrigation. In the hot wet season, Chomrong and Sahel202 showed significant differences between treatments at flowering (

Table 3), Chomrong developed the smallest leaf area in both treatments and Sahel202 had the largest leaf area. In the cold dry season, only Chomrong developed a significantly different leaf area between the treatments.

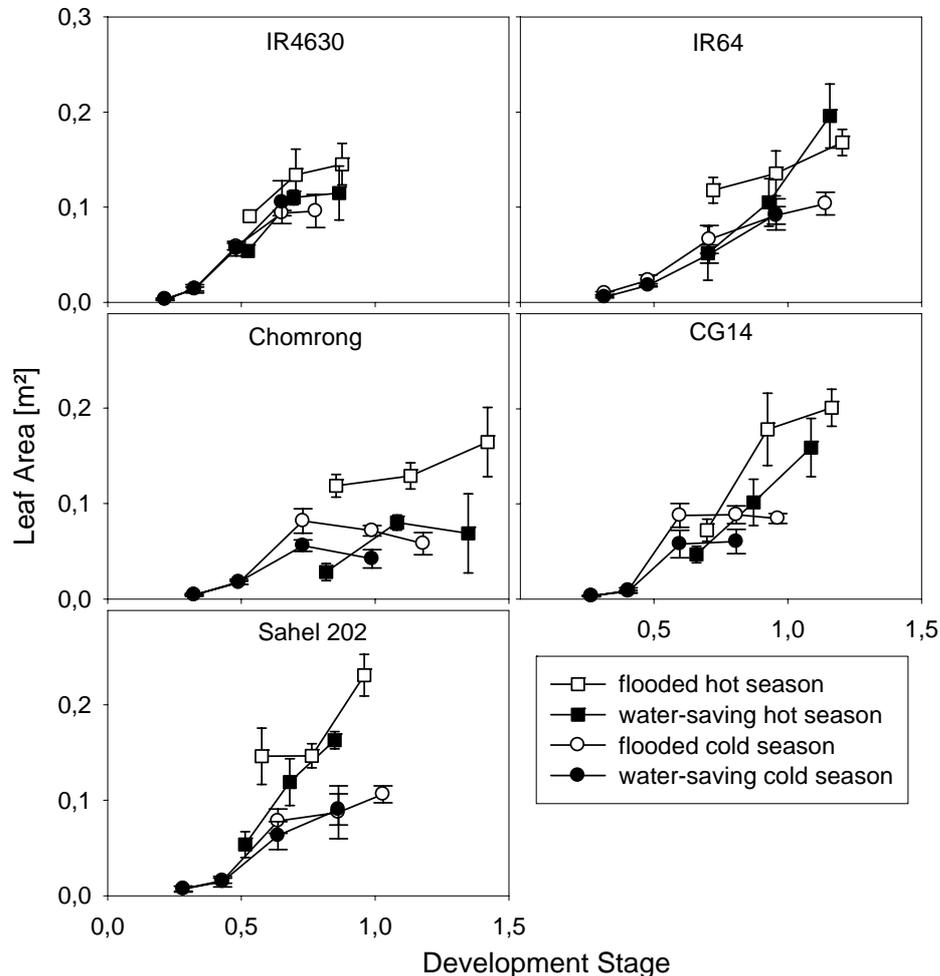


Figure 5 Leaf area of IR4630, IR64, Chomrong, CG14, and Sahel202 in hot wet season (square) and cold dry season (circle), flooded (white) and water-saving (black) treatment plotted against development stage of the plants (0=emergence, 1=flowering).

### 5.2.1.2 Seasonal pattern

Mean leaf area of individual hills is shown for two sowing dates and each variety in Table 3 (only PI and flowering) and shown as figure in Figure 12. Under conventional irrigation (flooded treatment) leaf area was higher in the hot wet season for all varieties but not always significant. IR64, CG14, and Sahel202 showed significant differences in leaf area between both seasons in the flooded treatment at flowering: leaf area in the cold season was 33 % (IR64), 65 % (CG14) and 52 % (Sahel202) smaller. In the water-saving treatment, CG14 and Sahel202 developed a significantly smaller leaf area (33 % and 44 % respective) in the cold season. Other leaf areas were not significantly different between seasons.

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*Table 3 Means of leaf area of 1 hill [m<sup>2</sup>] (curves fitted with TableCurve and development stages calculated with equation 1) for 5 varieties, two treatments (F= flooded, WS= water-saving) and two seasons, hot wet season (HS) and cold dry season (CS) are shown for panicle initiation (PI) and flowering, results of T-test ( $p \leq 0.05$ ) for comparison of treatments (small letters) and seasons (capital letters), values in one block with the different letters are significantly different.*

Variety	T	PI		Flowering	
		HS	CS	HS	CS
IR4630	F	0.10 a A	0.05 a A	0.12 a A	0.09 a A
	WS	0.08 a A	0.05 a B	0.11 a A	0.05 a A
IR64	F	0.10 a A	0.04 a A	0.15 a A	0.10 a B
	WS	0.06 a A	0.03 a A	0.17 a A	0.09 a A
Chomrong	F	0.09 a A	0.02 a B	0.12 a A	0.08 a A
	WS	0.02 b A	0.02 a A	0.07 b A	0.05 b A
CG14	F	0.08 a A	0.02 a B	0.2 a A	0.07 a B
	WS	0.06 a A	0.02 a B	0.15 a A	0.05 a B
Sahel202	F	0.11 a A	0.07 a A	0.23 a A	0.11 a B
	WS	0.08 a A	0.06 a A	0.16 b A	0.09 a B

### **5.2.1.3 Varieties**

Comparison of leaf area development (Table 3) also shows varietal differences. At flowering, leaf area of IR4630 amounted to 0.12 m<sup>2</sup> and 0.09 m<sup>2</sup> in the flooded treatment for the hot wet and cold dry season. In the water-saving treatment, leaf area in the hot season was 8 % lower than in the flooded treatment, whereas in the cold season, leaf area of the water-saving treatment was 44 % lower but not significant. IR64 showed slight higher leaf area, with non significant treatment differences.

Chomrong developed the lowest leaf area at flowering. Differences between treatments were significant in both seasons, with 42 and 38 % smaller leaf area in the water-saving treatment (hot season and wet season respective). Mainly in the hot season, CG14 developed a large leaf area which was not significantly different between seasons.

Sahel202 developed the largest leaf area in both seasons. Significant differences could be found between treatments and also between seasons.

### 5.2.1.4 Specific leaf area development

SLA development for both seasons and treatments is shown in Figure 6. In the hot wet season SLA was higher compared to the cold dry season over both treatments.

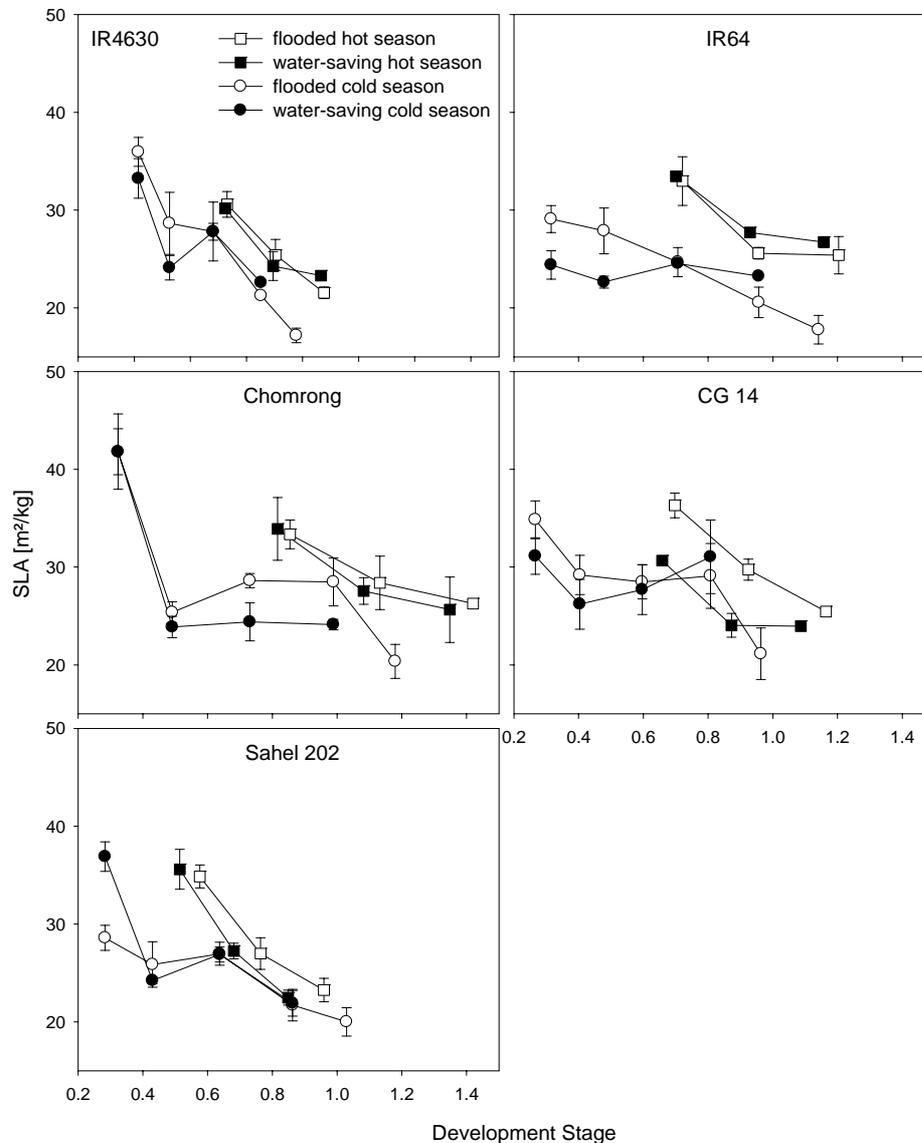


Figure 6 Specific leaf area of IR4630, Chomrong, Sahel202 (left) and IR64 and CG14 (right) for two seasons (hot wet season=square and cold dry season=circle) and flooded (white) and water-saving treatment (black) with development stages (1 = flowering), error bars show standard errors of means.

Differences in SLA between the treatments were small. In general SLA in the water-saving treatment tended to be slightly lower over all varieties and seasons. In the hot wet season, SLA in the water-saving treatment was only between 1 and 17 % smaller than in the flooded treatment.

On the contrary in IR4630, SLA in the water-saving treatment exceeded the SLA under flooded conditions up to 8 % (hot wet season). In the cold dry season SLA was up to 19 % lower under water-saving conditions before panicle initiation (PI); after PI it was up to 13 % higher.

In the hot wet season, the largest SLA was found for CG14 in the flooded treatment (36.3 m<sup>2</sup> kg<sup>-1</sup> near PI) which is significantly larger than IR4630 (smallest SLA with 30.6 m<sup>2</sup> kg<sup>-1</sup> around PI). In the water-saving treatment, Sahel202 had the largest (35.6 m<sup>2</sup> kg<sup>-1</sup>) and again IR4630 the smallest SLA (30.6 m<sup>2</sup> kg<sup>-1</sup>). Significant differences between treatments were only found for CG14 at 43 and 57 DAS (Table 4). At 71 DAS, there were no significant differences between treatments.

*Table 4 Means of SLA of all varieties in the hot wet season, 3 samplings and flooded (F) and water-saving (WS) treatment. Small letters indicate significant differences between treatments and capital letters indicate significant differences between varieties.*

	43 DAS		57 DAS		71 DAS	
	F	WS	F	WS	F	WS
IR4630	30.6 a A	30.2 a A	25.4 a A	24.7 a A	21.6 a A	23.3 a A
IR64	33.0 a AB	33.4 a AB	25.6 a A	27.7 a A	25.4 a AB	26.7 a A
Chomrong	33.3 a AB	33.9 a AB	28.4 a AB	27.6 a A	26.3 a B	25.8 a A
CG14	36.3 a B	29.5 b A	30.2 a B	23.9 b A	25.5 a AB	24.0 a A
Sahel202	34.9 a AB	35.6 a B	27.0 a AB	27.2 a A	22.4 a AB	22.5 a A

In the cold dry season, SLA showed no significant differences between treatments except Sahel202 at 29 DAS (Table 5). SLA near PI ranged from 24.7 m<sup>2</sup> kg<sup>-1</sup> (IR64) to 28.6 m<sup>2</sup> kg<sup>-1</sup> (Chomrong) in the flooded and 24.4 m<sup>2</sup> kg<sup>-1</sup> to 27.8 m<sup>2</sup> kg<sup>-1</sup> in the water-saving treatment.

Significant differences between varieties were found at 29 and 88 DAS. At 29 DAS in the flooded treatment, IR64 and Sahel202 had a significantly smaller SLA than Chomrong while the other varieties were intermediate. In the water-saving treatment, IR64 had a significantly smaller SLA than CG14 and Sahel202, which differed significantly from Chomrong (largest SLA). At 88 DAS, SLA of IR64 differed significantly from CG14 in the flooded treatment, whereas in the water-saving treatment, CG14 had a significantly higher leaf area than all other varieties.

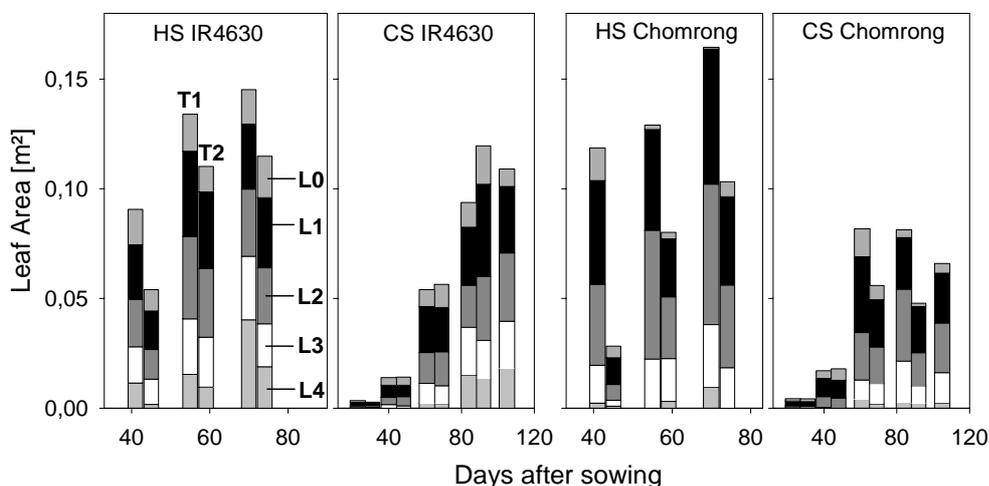
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*Table 5 Means of SLA of all varieties in the cold dry season, 3 samplings and flooded (F) and water-saving (WS) treatment. Small letters indicate significant differences between treatments and capital letters indicate significant differences between varieties.*

	29 DAS		44 DAS		65 DAS		88 DAS	
	F	WS	F	WS	F	WS	F	WS
IR4630	36.0 a BC	30.6 a AB	28.6 a A	24.1 a A	27.8 a A	27.8 a A	21.0 a AB	22.6 a A
IR64	29.1 a A	24.4 a A	27.9 a A	22.6 a A	27.6 a A	24.5 a A	19.9 a A	23.2 a A
Chomrong	41.8 a C	44.4 a C	25.4 a A	23.9 a A	28.6 a A	24.4 a A	28.5 a BC	24.1 a A
CG14	34.8 a AB	36.7 a B	29.2 a A	26.2 a A	28.5 a A	27.7 a A	29.1 a C	31.0 a B
Sahel202	28.6 a A	36.9 b B	25.9 a A	24.2 a A	27.0 a A	26.9 a A	21.7 a AB	21.9 a A

## 5.2.2 Leaf area development of leaf levels

Depending on their position on the stem, leaves are assigned to different leaf levels. Leaf area of the entire plant can be split up into the leaf area of the distinct leaf levels. In Figure 7 the distribution of leaf area among leaf levels is shown for two varieties.



*Figure 7 Distribution of leaf area among the leaf levels. Two varieties as examples: IR4630 (left) and Chomrong (right), each shown for hot wet season (HS) on the left and cold dry season (CS) on the right side, T1=flooded, left bar, T2=water-saving, right bar, youngest fully developed leaf=L1, L2-L4=following older leaves, L0=not yet fully developed leaves*

The area of every leaf level increased until flowering (increase stopped earlier for L1 and lasted longest time for L4), afterwards it was relatively stable. L1 had usually the largest leaf area of all leaf levels. The five varieties can be divided into two groups

which showed similar results. The first group includes IR4630, IR64 and Sahel202. In the hot wet season, the proportion of the first leaf level was 26 % in the flooded and 32 % in the water-saving treatment, for the cold dry season it was 34 % and 37 % respectively. The second group includes Chomrong and CG14, with an area of the first leaf level of 36 % or 33 % (hot wet season, flooded and water-saving) and 42 % for both treatments in the cold dry season. L2 of IR6430, IR64 and Sahel202 had an area of 21 to 35 % of the entire plant. Chomrong and CG14 had a bigger range of leaf area L2 with 18 to 46 %.

### 5.2.2.1 Specific leaf area of leaf levels

Specific leaf area of the distinct leaf levels showed similar results for all varieties. Figure 8 shows the SLA of the 5 leaf levels of IR4630.

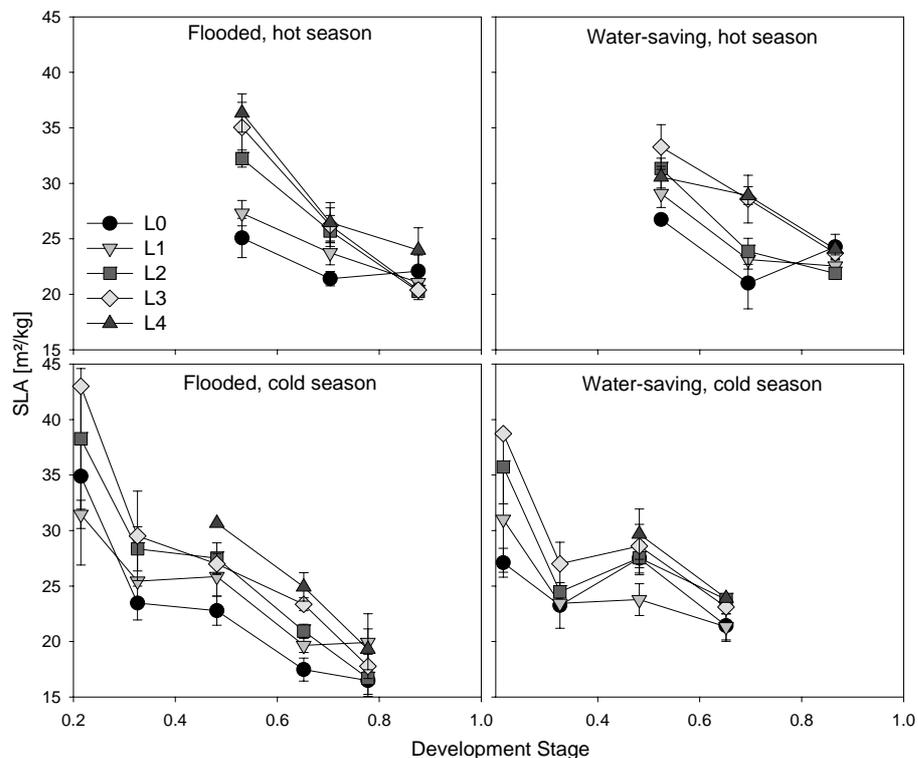


Figure 8 Specific leaf area development of all leaf levels of IR4630 under flooded (left) and water-saving (right) conditions in two seasons: hot wet season at the top and cold dry season below, youngest fully developed leaf=L1, L2-L4=following older leaves, L0=not yet fully developed leaves, development stage 0=emergence, 1=flowering, error bars show standard error of means.

As for the whole plant, SLA started at a high level and declined during development. SLA of the leaf levels followed a clear regularity: the lowest SLA was found for the youngest leaves, L0 or L1, depending on the development stage of L0, while the

highest SLA was found for the oldest leaf of every culm (L4). IR4630, as an example, had a range of SLA between  $25.1 \text{ m}^2 \text{ kg}^{-1}$  (L0) and  $36.3 \text{ m}^2 \text{ kg}^{-1}$  (L4) close to PI (43 DAS) and  $22.1 \text{ m}^2 \text{ kg}^{-1}$  and  $24.0 \text{ m}^2 \text{ kg}^{-1}$  close to flowering (71 DAS) in the flooded treatment in the hot wet season. In the cold dry season SLA of the youngest leaf level was  $34.9 \text{ m}^2 \text{ kg}^{-1}$  and  $43.0 \text{ m}^2 \text{ kg}^{-1}$  for the oldest leaf level at 43 DAS; at 71 DAS it was  $16.5 \text{ m}^2 \text{ kg}^{-1}$  and  $19.9 \text{ m}^2 \text{ kg}^{-1}$  respectively. SLA of the entire hill was higher than SLA of leaf levels L0 and L1 and lower than the other leaf levels.

### 5.2.2.2 Specific leaf area development of single leaves

SLA of single leaves was not stable but developed over leaf age. SLA development is shown in Figure 9 for IR4630 under flooded conditions in the hot wet season. Samples were taken from leaf 8 to 15. To compare the SLA during leaf development, leaf development stages from 0, which represents the time of appearance of the leaf, to 2 (leaf death) were used. Leaf 8, as the first measured leaf, had the highest SLA of all measured leaves. The later the leaves appeared during plant development, the smaller was the SLA and also the slope of SLA development of one single leaf. From leaf 14, the leaf before the flag leaf, SLA was constant.

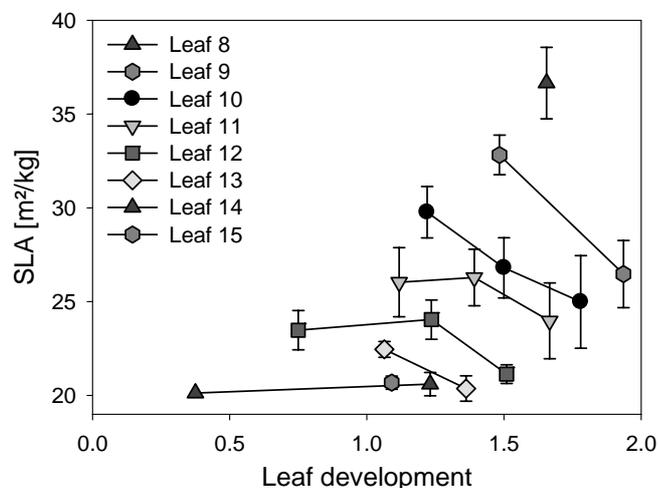


Figure 9 Development of SLA of single leaves (8-15 on the main culm) of IR4630, in flooded treatment of hot wet season. Leaf development 0 = leaf appearance, 1 = fully developed leaf, 2 = leaf senescence, error bars show standard error of means.

### 5.2.2.3 Using SLA of distinct leaf levels to estimate the leaf area of the entire hill

In order to establish a method to estimate leaf area of the whole hill without measuring every single leaf, SLA of the different leaf levels was used to calculate the entire leaf area and results were compared to measured leaf area. Leaf area was calculated with

SLA of leaf levels in all combinations and leaf dry weight of the entire hill. An example is shown in Figure 10.

The measured leaf area of the hill is taken as reference value (zero-line). Depending on the choice of leaf levels, calculated leaf area can show a large deviation from measured results. Deviation, expressed in percentage, differed for the different samplings. At the first sampling (43 DAS), all calculated leaf areas overestimated the measured leaf area, except L1, which underestimated the measured leaf area by about 3 %. The best fit gave leaf level L1+2 (a combination of leaf levels 1 and 2), which was only 1 % higher than the measured leaf area of the whole hill. Sampling around flowering showed a greater variation. The best fit was found for leaf level L1+2+3, which underestimated the leaf area of the hill by 0.3 % only. At 71 DAS, calculations based on the upper leaf levels underestimated the leaf area while calculations based on the lower leaves led to overestimation. Best estimation was achieved with L1+2+3+4, which overestimated leaf area about 0.4 %. Leaf levels 3 and 4 and the combination of the two usually led to the poorest fit with the measured leaf area. In case of IR64 (Figure 10), leaf level 3 over- or underestimated leaf area by 3 to 17 %, leaf level 3+4 by 2 to 15 % and leaf level 4 by 8 to 13 %.

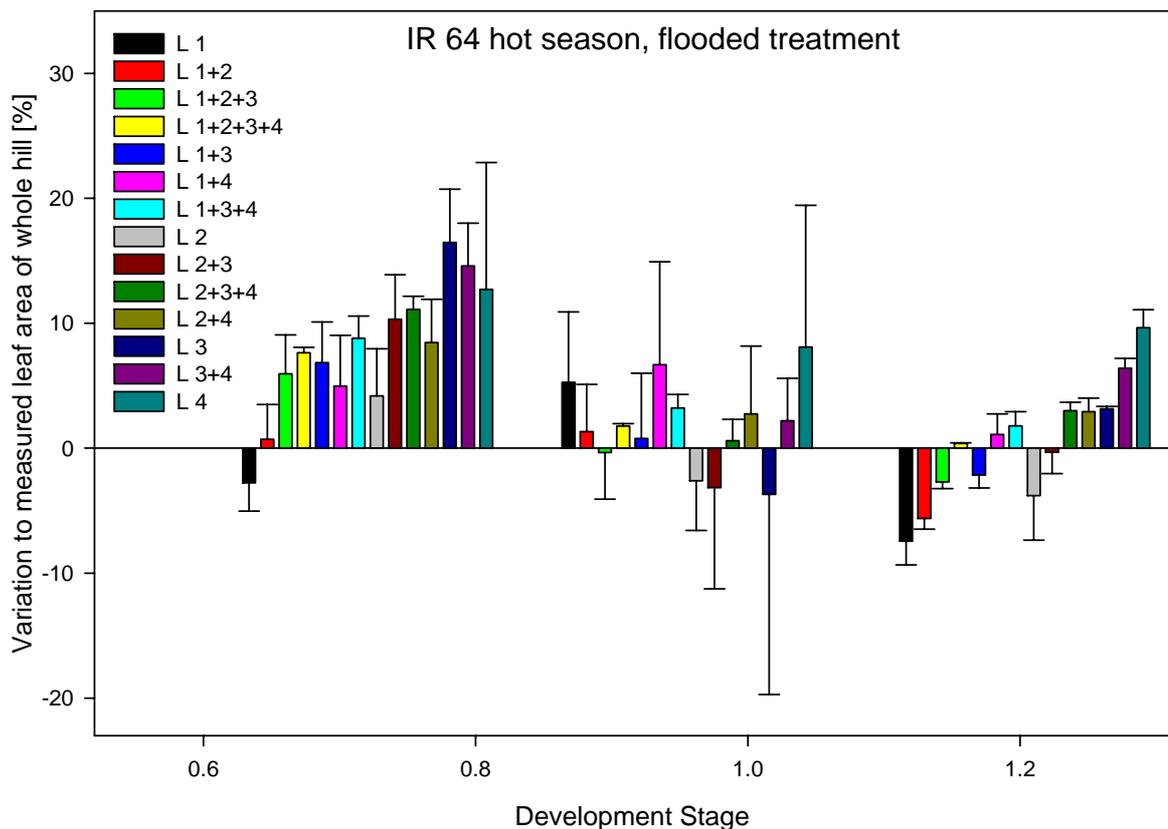


Figure 10 Difference of leaf area of all combinations of leaf levels to measured leaf area of the whole hill (=zero-line), shown for flooded treatment of hot wet season of IR64. L1 means only leaf level 1, while L1+2 stands for leaf area calculated with leaf levels 1 and 2 (always counted from the top) etc.

In Figure 11, the calculated deviations of all varieties and both sowing dates and treatments were averaged and plotted for all leaf levels. Over all varieties, treatments and sowing dates, leaf area estimation on the basis of leaf level 1+2 resulted in the best approximation to the measured leaf area (4.5 %). The best approximation including only one leaf level was L2 which had an average deviation of 7 %. Leaf level L1 which is used in the RISOCAS trial to estimate leaf area via SLA without measuring the entire hill had an average deviation of 8 %.

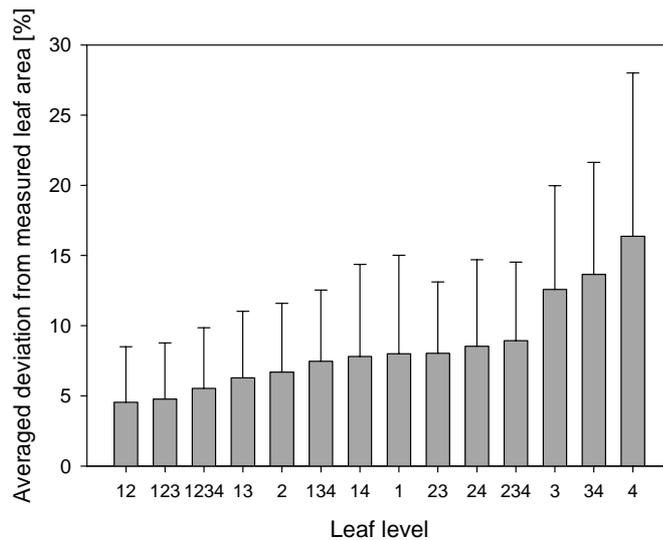


Figure 11 Averaged deviation of leaf area over all seasons, treatments and varieties, calculated for various leaf levels from measured leaf area of the whole hill (zero-line), L1 means only leaf level 1, while L12 stands for leaf area calculated with leaf levels 1 and 2 (always counted from the top) etc.

### 5.2.3 Validation of a method for estimating leaf area

Leaf area in the RISOCAS-trials was estimated by measuring leaf area and dry weight of the youngest fully developed leaf (L1) of the main tiller followed by SLA calculation. Leaf dry weight of the entire hill was determined and multiplied by the SLA of L1 for leaf area.

Figure 12 shows measured leaf area of this study (squares) and estimated leaf area of RISOCAS (as big circles) and all the leaf areas that were estimated with different leaf levels (little circles). The leaf area that was calculated in RISOCAS trial usually underestimates the measured leaf area of the entire hill.

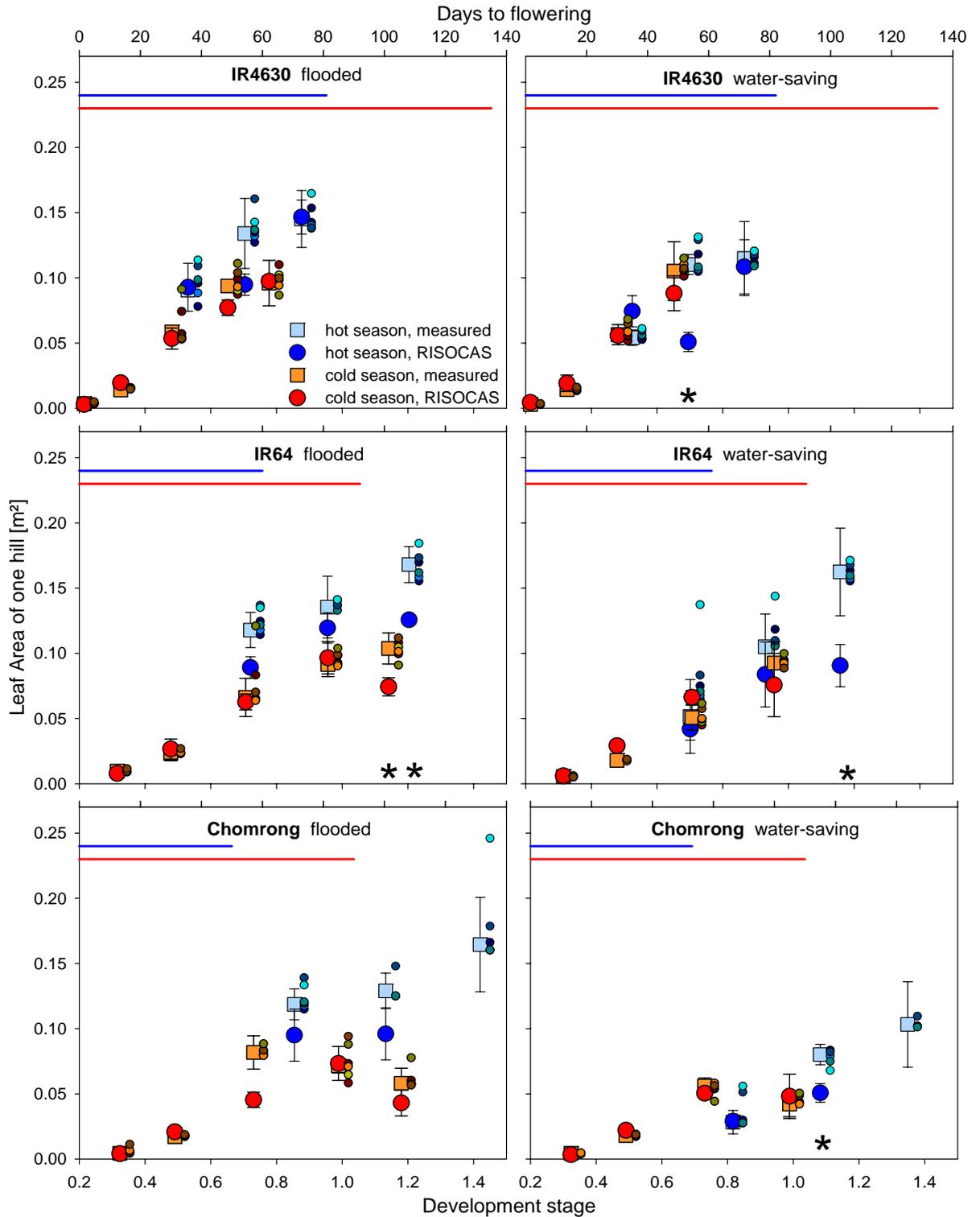


Figure 12 Development of leaf area (measured LA shown as squares) of IR4630, IR64 and Chomrong in two treatments (left flooded and right water-saving treatment) and two seasons (hot wet season = blue, cold dry season = red). Calculated leaf area from the RISOCAS trial is shown as circles, small circles show the range of leaf area calculated with SLA of different leaf levels. Duration from sowing to flowering is shown as blue lines for hot wet season and red lines

for cold dry season. Error bars show standard error of means, \* indicate significant values from *t*test ( $p \leq 0.05$ ).

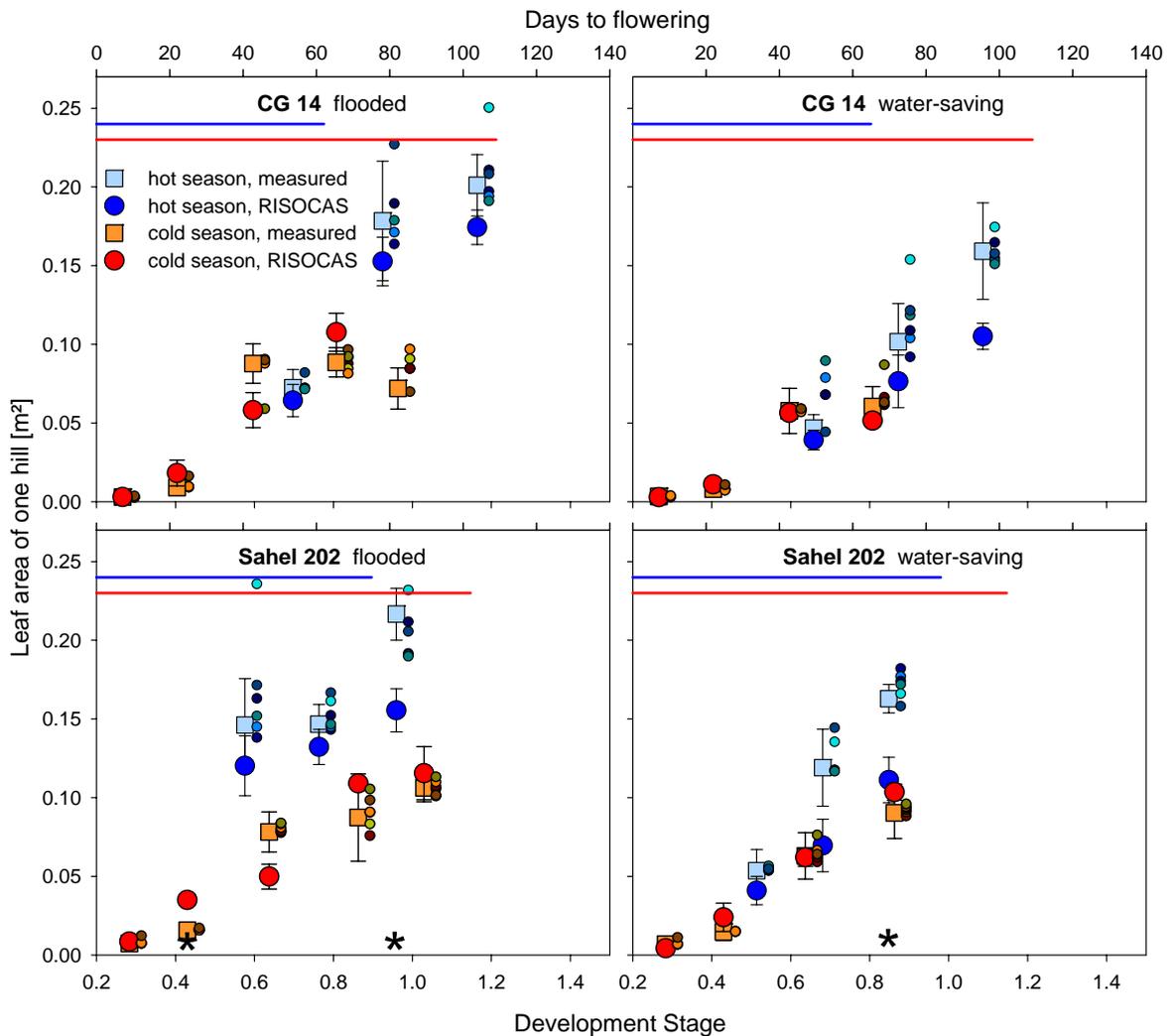


Figure 12 (continued) Development of leaf area (measured LA shown as squares) of CG14 and Sahel202 in two treatments (left flooded and right water-saving treatment) and two seasons (hot wet season = blue, cold dry season = red). Calculated leaf area from the RISOCAS trial is shown as circles, small circles show the range of leaf area calculated with SLA of different leaf levels. Duration from sowing to flowering is shown as blue lines for hot wet season and red lines for cold dry season. Error bars show standard error of means, \* indicate significant values from *t*test ( $p \leq 0.05$ ).

In the hot wet season, IR64 and Sahel202 showed significant differences between the different calculation methods near flowering regardless of treatments. Chomrong showed a significant smaller leaf area for calculated leaf area in the RISOCAS trial in the water-saving treatment; however the last sampling was not carried out due to

severe rat damage. Before flowering, only IR4630 under water-saving irrigation had a significantly larger leaf area (measured) at one sampling, but this is probably due to scanning mistake in the RISOCAS trial. Leaf area of CG14 was not significantly different for all samplings in the hot wet season. Over all development stages, leaf area of the RISOCAS trial was from 1 % larger (IR4630) to 33 % smaller (Sahel202) in the flooded and 6 % (IR4630) to 37 % (Chomrong) smaller in the water-saving treatment compared to the measured leaf area.

In the cold dry season, the only significant difference was found for IR64 in the flooded treatment at flowering. Differences between measured and estimated values ranged between 4 % and 11 % (Sahel202 and IR4630) under flooded and between -0.6 % and 28 % (Chomrong and IR64) under water-saving conditions.

## **5.3 Transpiration**

### **5.3.1 Transpiration of single leaves of the main tiller**

Transpiration of leaves of the main culm was measured in hot wet and cold dry season in both treatments for IR4630 and IR64.

Transpiration rates of leaf levels measured on the main culm of IR4630 and IR64 in hot wet season are shown in Figure 13. Measured values for transpiration ranged between 3 mmol m<sup>-2</sup>s<sup>-1</sup> (water-saving treatment, IR64) and 11 mmol m<sup>-2</sup>s<sup>-1</sup> (flooded treatment, IR4630). Transpiration rate differed between varieties, for IR4630 it was larger than for IR64. Flooded conditions enhanced transpiration compared to the water-saving treatment.

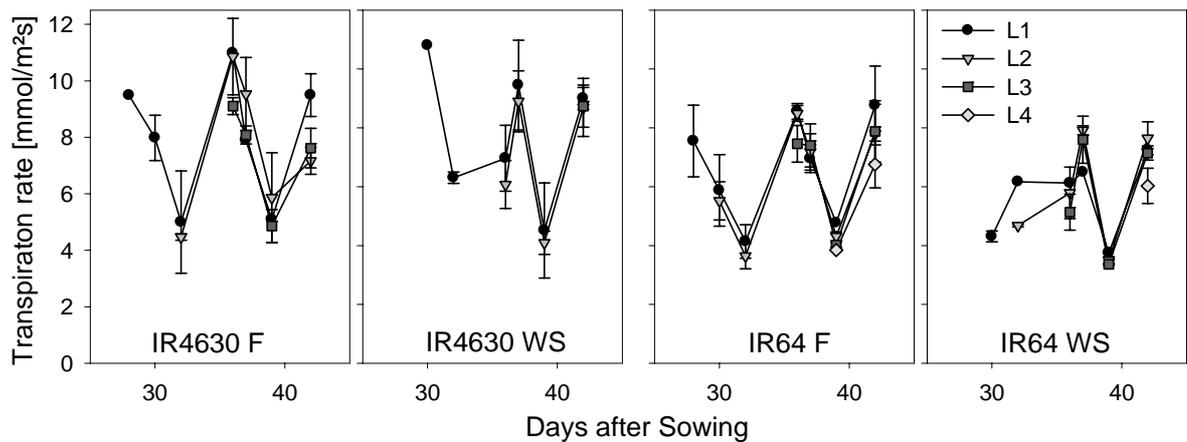


Figure 13 Transpiration rate in the hot wet season of IR4630 (left) and IR64 (right) in flooded (F) and water-saving treatment (WS). L1 stands for leaf level of the youngest fully developed leaf, and L2 to L4 were the following older leaves. Error bars show standard error of means.

Averaged transpiration rate of the flag leaf was  $8 \text{ mmol m}^{-2}\text{s}^{-1}$  for IR4630 (flooded) and  $7.8 \text{ mmol m}^{-2}\text{s}^{-1}$  (water-saving), in IR64 transpiration rates of  $6.7$  and  $5.7 \text{ mmol m}^{-2}\text{s}^{-1}$  were measured. With regard to leaf levels, differences in transpiration between measured leaves were small.

Transpiration rates of the five leaf levels of IR4630 and IR64 in both treatments are shown in Figure 14 for the cold dry season.

These transpiration rates (Figure 14) were overall smaller than those of the hot wet season (Figure 13). In IR4630, average transpiration rates were  $5.8$  and  $4.6 \text{ mmol m}^{-2}\text{s}^{-1}$  in the flooded and water-saving treatment, which was 28 and 22 % smaller compared to the correspondent results in the hot wet season. Average transpiration rates of IR64 during the cold dry season ( $5.2 \text{ mmol m}^{-2}\text{s}^{-1}$ , flooded and  $4.8 \text{ mmol m}^{-2}\text{s}^{-1}$ , water-saving) were 41 and 16 % lower compared to the hot wet season.

The highest ( $8.4 \text{ mmol m}^{-2}\text{s}^{-1}$ ) and lowest ( $0.3 \text{ mmol m}^{-2}\text{s}^{-1}$ ) transpiration rate in cold dry season was both found in IR4630.

In the flooded treatment, no differences between varieties were found (Table 6). In the water-saving treatment, IR4630 developed a significantly higher transpiration rate than IR64.

From beginning of measurements to 54 days after sowing (DAS) and from 85 DAS, IR4630 had higher transpiration rates than IR64. Between 55 and 84 DAS, IR64 developed higher transpiration rates in the flooded treatment, while there were only

small varietal differences in the water-saving treatment. Near PI (80 DAS), the youngest fully developed leaf of IR4630 showed a transpiration rate of  $7.0 \text{ mmol m}^{-2}\text{s}^{-1}$  while the transpiration rate of IR64 was  $5.0 \text{ mmol m}^{-2}\text{s}^{-1}$  (56 DAS).

*Table 6 Means of transpiration rate compared for treatments and varieties, small letters indicate significant differences between varieties and capital letters indicate significant differences between treatments. Measurement dates were only taken into account when all leaf levels were present.*

Treatment	IR4630	IR64
flooded	5.3 a A	5.2 a A
water-saving	5.3 a A	4.6 b B

Transpiration rate was usually higher in the flooded treatment. Under flooded conditions, IR64 had significant higher transpiration rates as compared to the water-saving treatment (Table 6). The differences in transpiration rate of IR64 (leaf level of the youngest fully developed leaf) between flooded and water-saving treatment ranged between 4 and 46 % over time. Before 60 DAS and after 80 DAS, transpiration rate of IR4630 was up to 27 % higher in the flooded treatment, whereas transpiration was 25 % higher in the water-saving treatment between 60 and 80 DAS. Overall, there were no significant differences found between treatments for IR4630.

In the beginning of the measuring period, only small differences between the leaf levels were observed. Differences between the highest and lowest leaf level became more important during plant development. The greatest difference between lowest leaf level and youngest fully developed leaf level was observed in the flooded treatment of IR64 with a difference of  $4.7 \text{ mmol m}^{-2}\text{s}^{-1}$ . The greatest difference in the flooded treatment of IR4630 was  $4.6 \text{ mmol m}^{-2}\text{s}^{-1}$ , for the water-saving treatment the differences were  $3.9 \text{ mmol m}^{-2}\text{s}^{-1}$  and  $3.8 \text{ mmol m}^{-2}\text{s}^{-1}$  for IR4630 and IR64 respectively.

Transpiration rate varied also between days. In Table 7 transpiration rates of measurement dates with all leaf levels present were compared. Three groups of days could be found which developed significant differences between each other.

*Table 7 Means of transpiration rate compared measurement days; small letters indicate significant differences between days. Measurement dates were only taken into account when all leaf levels were present.*

Date	Mean
05.01.2010	6.03 a
31.12.2009	5.97 a
08.01.2010	5.11 b
04.01.2010	4.71 b
14.01.2010	4.59 bc
12.01.2010	4.12 c

Figure 14 also illustrates floating averages of both varieties and seasons. Floating averages were used to observe long term effects and overall differences in the strongly fluctuating data set. Floating averages showed larger differences between leaf levels in the flooded treatment for both varieties, the youngest fully developed leaf had the highest transpiration rates, e.g. for IR64, floating average of transpiration at 72 DAS reached  $8.2 \text{ mmol m}^{-2}\text{s}^{-1}$ . The first leaf level was followed by leaf level 2 and 3 (following deeper leaves), that differed only marginally (with  $6.7$  and  $6.6 \text{ mmol m}^{-2}\text{s}^{-1}$  respective). Within the lower leaf levels, transpiration rate was decreasing. Levels 4 and 5 showed transpiration rates of  $6.0$  and  $4.8 \text{ mmol m}^{-2}\text{s}^{-1}$  respectively.

From 53 to 67 DAS, leaf level 2 had up to  $1.2 \text{ mmol m}^{-2}\text{s}^{-1}$  higher transpiration rates than leaf level 1 in the water-saving treatment of IR4630. From 71 DAS, leaf level 3 had mostly the highest transpiration rates. Transpiration of leaf level 3 was at the highest point  $1.9 \text{ mmol m}^{-2}\text{s}^{-1}$  higher than transpiration of leaf level 1.

*Table 8 Means of transpiration rate compared for treatments and leaf levels (counted from the top, L1 = youngest fully developed leaf), small letters indicate significant differences between treatments and capital letters indicate significant differences between leaf levels. Measurement dates were only taken into account when all leaf levels were present.*

Leaf level	flooded	water-saving
1	6.1 a A	5.0 b A
2	5.6 a AB	5.2 a A
3	5.1 a B	5.1 a A
4	4.1 a C	4.5 a A

Mean transpiration rates of both varieties decrease in the flooded treatment from highest to the lower leaves and there are statistically significant differences (Table 8). In contrast, leaf levels of the water-saving treatment developed nearly similar transpiration rates from leaf levels 1 to 4 which were not significantly different. The only significant difference between treatments was found for leaf level 1, for the lower leaf levels, transpiration rates were not significantly different.

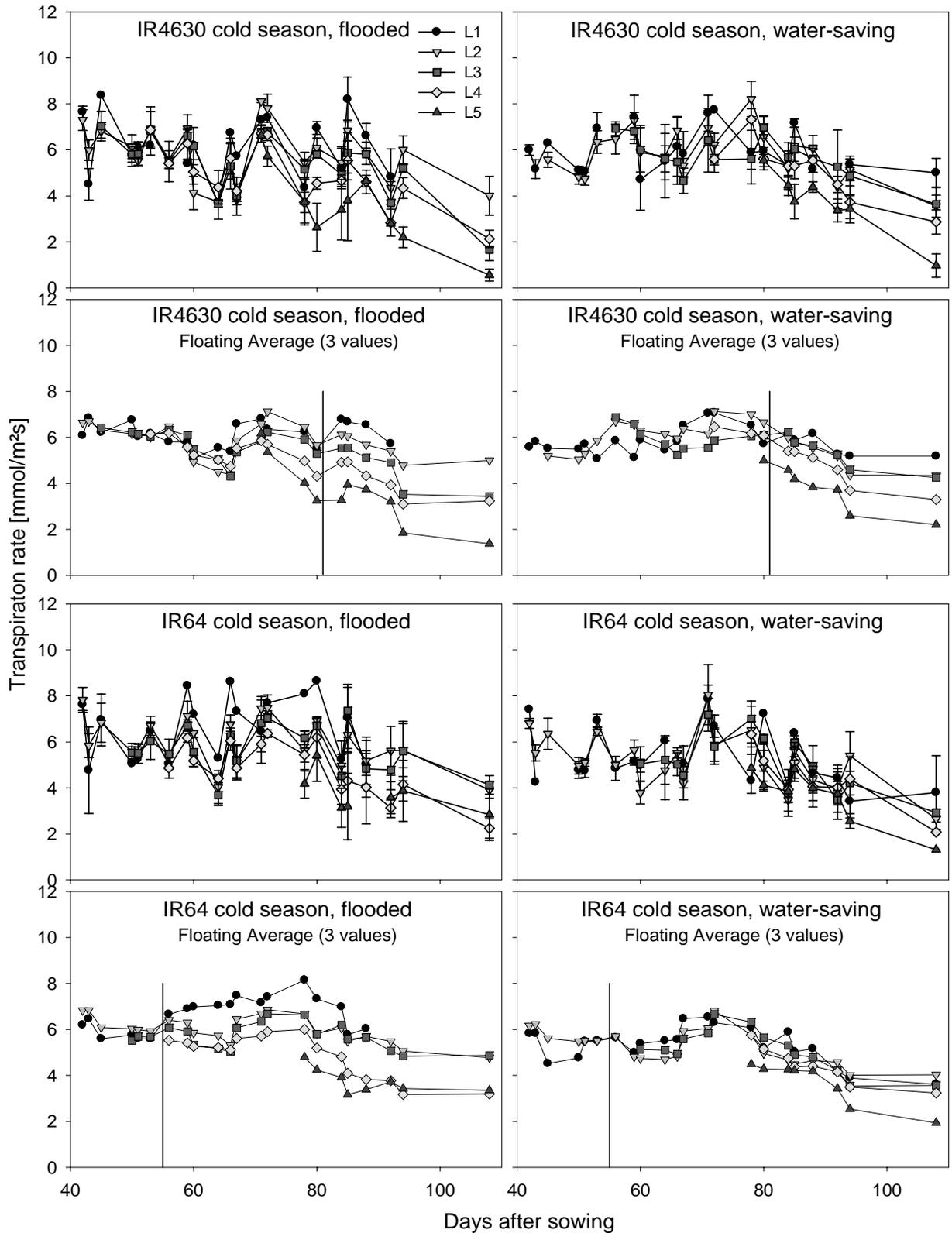


Figure 14 Transpiration rate in the cold dry season of IR4630 (above) and IR64 (below) in flooded (left) and water-saving treatment (right). L1 = youngest fully developed leaf, L2 to L4 = following older leaves, corresponding floating average of each level is shown below. Error bars show standard error of means, vertical lines represent panicle initiation (PI).

The comparison of transpiration rates as floating averages of single leaves over time is presented in Figure 15 for leaves 9 (left side) to 12 (right side). In IR64, flooded and water-saving treatment of leaf 9 differed less than 10 %. Larger differences were observed in leaf level 9 of IR4630, with up to 44 % higher transpiration rates around PI (80 DAS) in the water-saving treatment compared to the flooded treatment. A big difference was observed between flooded and water-saving treatment of leaf 10 of IR4630, where transpiration rate in the flooded treatment was 34% lower. Leaf 11 of IR4630 in the water-saving treatment developed higher transpiration rates than the other leaves number 11. Regarding leaf 12, transpiration of the flooded treatment of IR4630 was less than in IR64 until 70 DAS; afterwards there were only small differences.

Transpiration rates increased with higher leaf numbers. The highest transpiration rates in IR4630 under water-saving irrigation were  $6.7 \text{ mmol m}^{-2}\text{s}^{-1}$ ,  $6.9 \text{ mmol m}^{-2}\text{s}^{-1}$ ,  $7.3 \text{ mmol m}^{-2}\text{s}^{-1}$  and  $8.5 \text{ mmol m}^{-2}\text{s}^{-1}$  (leaf 9 to 12).

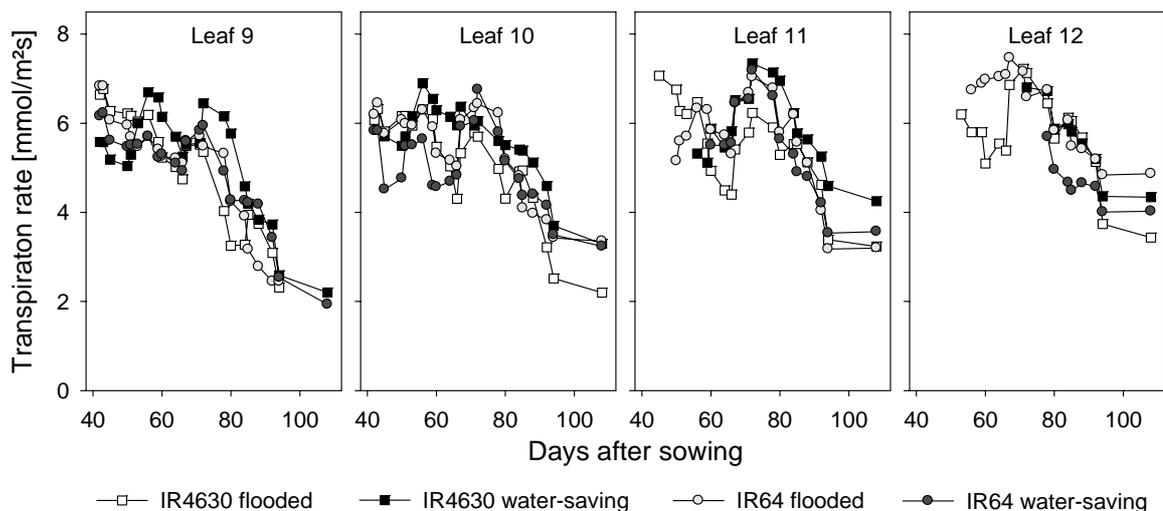


Figure 15 Floating averages of transpiration rate from leaf 9 (left side) to 12 (right side) of IR4630 (square) and IR64 (circle), flooded treatment (white) and water-saving treatment (dark)

### 5.3.2 Diurnal gas exchange of leaves on the main tiller

Diurnal gas exchange was measured once a week with at least four to five measurements per plant and day. Development of transpiration rate within one day is shown as example for both treatments of IR64 in Figure 16 for two contrasting days.

In the morning, transpiration rate started low and increased to a maximum transpiration value which was usually measured between 1 and 2 p.m. After this peak, transpiration rate decreased.

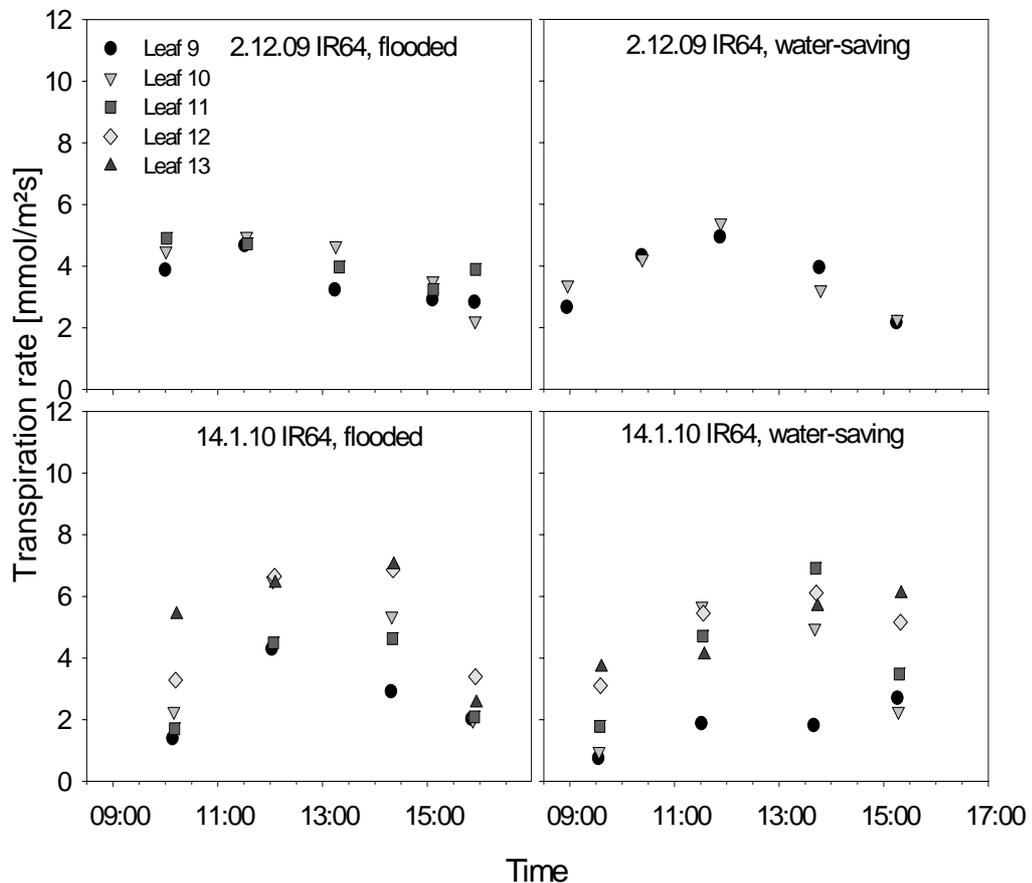


Figure 16 Diurnal transpiration rates of one replication of IR64, flooded treatment (left) and water-saving treatment (right) during two contrasting days: 2.12.09: cloud cover, cold, no wind, 14.1.10: sunny, windy, warm with high temperature amplitude.

Single leaves followed the same pattern as leaves which were measured once a day: Youngest fully developed leaves usually showed the highest transpiration rates. Lower leaves showed lower transpiration rates.

Only small differences were observed between the shape of transpiration curves of flooded and water-saving treatment (Figure 16). In general, transpiration rates of plants in the flooded treatment were slightly higher as compared to the water-saving treatment.

Shapes of diurnal transpiration curves differed under different weather conditions. Transpiration rate on cold and cloudy days was lower, with little differences between leaf levels and a low peak. Sunny and windy days led to higher transpiration rates around noon and higher variation of transpiration of the leaf levels.

### 5.3.3 Estimation of transpiration over the cropping period

Daily transpirational water loss of every leaf level was cumulated in consideration of the specific weather patterns (Figure 17). Transpiration of the youngest fully developed leaf level always contributed the biggest share of the total transpiration, which amounted to between 79.1 mm and 104.4 mm at 105 DAS, and was followed by transpiration of leaf level L2.

In the flooded treatment, the transpiration of leaf level L3 and L4 showed bigger differences (e.g. IR64: 60.3 mm for leaf level 3 and 32.8 mm for leaf level 4), whereas L3 and L4 were in the same range in the water-saving treatment (34.3 mm and 32.0 mm respective). Plants in the flooded treatment showed a larger transpiration. Transpiration of IR4630 was 12 % greater in the flooded treatment; IR64 had a 31 % higher transpiration for the first 105 days after sowing.

Different treatments led to different transpiration among varieties. The first 105 days after sowing, IR4630 transpired 9 % less water than IR64 in the flooded treatment but under water-saving conditions, it transpired 14 % more.

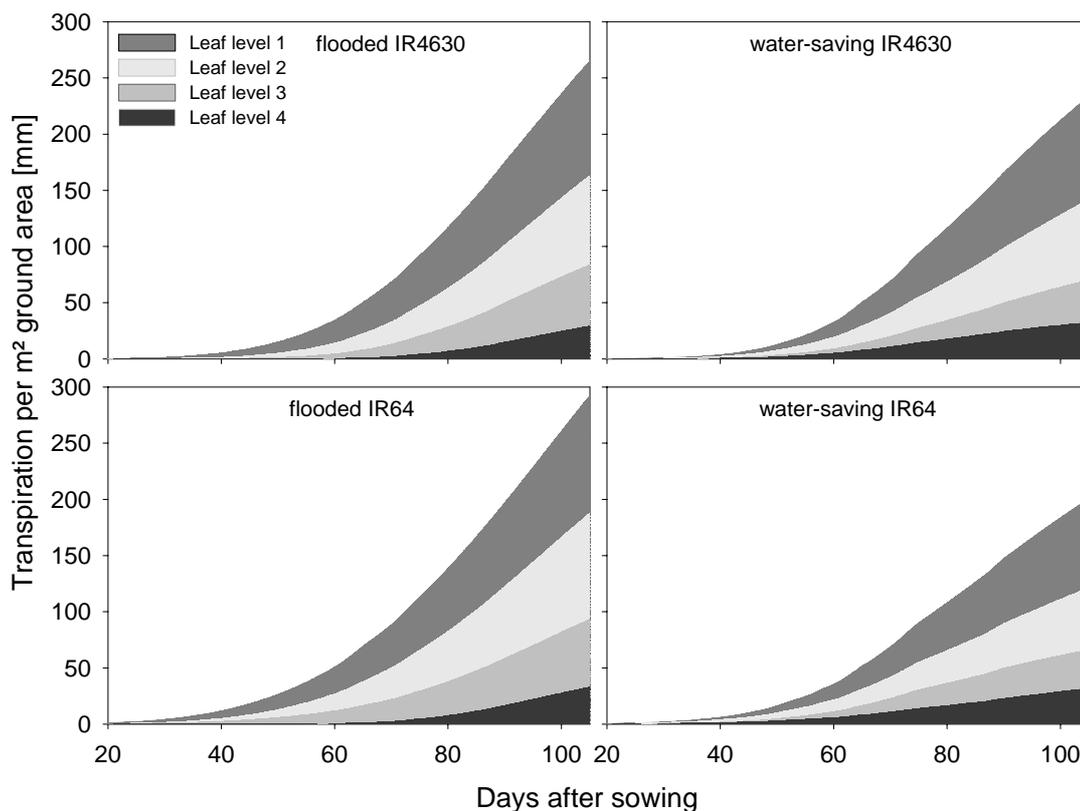


Figure 17 Cumulated transpiration per unit ground area of IR4630 (above) and IR64 (below) in the flooded (left) and water-saving (right) treatment, for the first 105 days after sowing in the cold dry season. Total transpiration is subdivided into transpiration of leaf levels (different colours).

Cumulated transpiration with shares of leaf levels is compared to calculated transpiration of the RISOCAS trial in Figure 18. Averaged shares of the leaf levels at total transpiration were 38 %, 30 %, 19 % and 13 % (leaf level L1 to L4) at 105 DAS. The share of leaf level L1 and L4 was higher in the water-saving treatment (L1: averaged 37 % flooded and 39 % water-saving), leaf level L2 and L3 was smaller (L2: averaged 31 % flooded and 29 % water-saving and 21 % and 17 % for leaf level L3 respective) in the water-saving treatment.

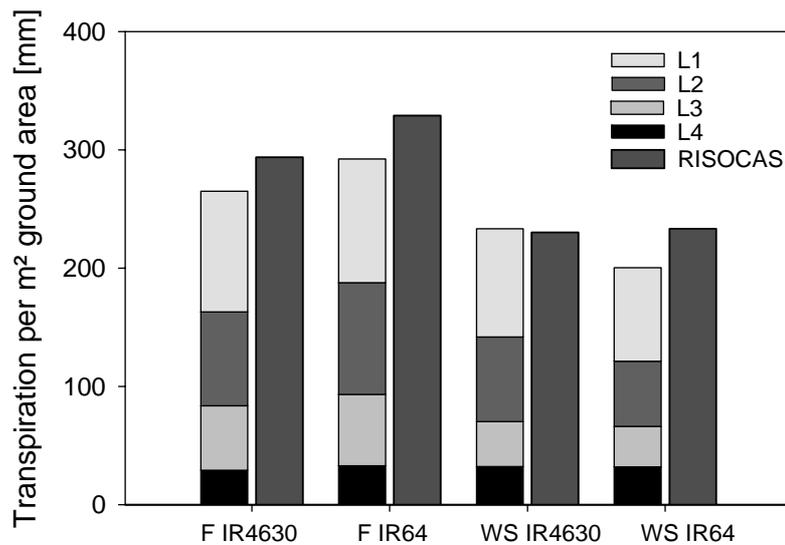


Figure 18 Comparison of calculated transpiration in the RISOCAS trial and the estimated transpiration (subdivided in shares of the different leaf levels (L1 to L4)) for flooded (F) and water-saving (WS) treatment of IR4630 and IR64 and the first 105 days after sowing.

Cumulated transpiration calculated with ORYZA2000 in the RISOCAS trials was higher than the cumulated transpiration estimated with measured values of this study. The estimated transpiration was 10 to 14 % smaller in both flooded and in the water-saving treatment of IR64. Only in the water-saving treatment of IR4630 estimated transpiration was slightly larger than calculated one.

## **6 Discussion**

### **6.1 Effects of season and water-saving irrigation**

#### **6.1.1 Effects on phyllochron**

Germination was delayed in the cold season. This is confirmed by findings of de Vries et al. (2010), who stated low night temperatures as reason. Leaf appearance (from leaf 9) was delayed in the water-saving treatment and in the cold season, before leaf 9, no differences were observed (Figure 3 and 4). Leaf duration was longer in the cold season, differences were observed between treatments but not between IR4630 and IR64. These findings fit to the work of Nguyen et al. (2009) who found a delay of 13 days in the water-saving treatment (SSC) and a general delay of phenological development through water stress (also stated by Davatgar et al. 2009).

The first leaves of rice sown in the cold season grew in a still warm environment. As the cold season proceeded, differences in leaf development between varieties became smaller. Differences between the treatments were expected due to the temperature buffering function of the standing water in the flooded treatment. Without standing water in the water-saving treatment, the apical meristem is subjected to larger temperature differences (Nguyen et al. 2009) which can lead to a delayed phyllochron.

In the hot season, varietal differences in leaf duration were observed. IR64 has a faster leaf development than IR4630 which is due to the crop duration of the varieties. Temperature variations between day and night were not as pronounced in the hot as in the cold season, buffering function of water is not needed and this is reflected in the small differences between treatments.

The biggest problem in determining leaf development was rat damage. Near harvest during the hot wet season, rats cut hills of the short duration varieties in the water-saving treatment which led to data loss of IR64. Final leaf number of the main culm of IR64 is not definitely known. In the cold dry season rats destroyed almost the entire plots, mainly in the water-saving treatment. Complete data loss of all replications of one variety could be prevented with little cages around single hills.

#### **6.1.2 Effects on leaf area and SLA**

Rice hills in the cold season developed smaller leaf areas and lower SLAs as compared to the hot wet season (Table 3, Figure 6). In the water-saving treatment, rice hills developed a smaller leaf area and SLA also tended to be lower. The same result

was found by Farooq et al. (2010) who stated that drought substantially decreased leaf area and SLA in IR64. Nguyen et al. (2009) also found reduced leaf area in Saturated Soil Culture treatments compared to flooded treatments and Borrell et al. (1997) explained the smaller leaf area with less tillers under water-saving conditions. Davatgar et al. (2009) stated that inhibition of leaf cell expansion or division by water stress were reasons for decreased leaf area in water-saving treatments and considered leaf area development to be more sensitive to water deficits than transpiration rate. In the flooded treatment, leaf area differences between seasons were more distinct. IR64 developed a smaller leaf area than IR4630.

The leaf level of the youngest fully developed leaf has a large share of total leaf area for CG14 and Chomrong and a smaller one for IR4630, IR64 and Sahel202 (Figure 7). Dingkuhn et al. (1992) also found most of the leaf area in the 1st and 2nd leaf positions beneath the flag leaf.

SLA develops over the development of a single leaf (Figure 9), starts high and declines, for later appearing leaves it starts a little smaller respectively. The youngest leaves of the hill have the smallest SLA. As previously reported by Dingkuhn et al. (1998), SLA was found to be strongly related to leaf area.

Leaf area and SLA development depend both on temperature (Tardieu et al. 1999; Dingkuhn et al. 2001). In the hot season, leaves grow faster and overall development is accelerated (Figure 3 and 4). SLA is larger in the hot season which corresponds with thinner leaves. When plant growth is accelerated, the plants have less time to accumulate starch and cellulose into the leaves which results in thinner leaves in a warm environment. Development of SLA of single leaves strongly depends on the age of the respective rice plant. The first leaves have a high SLA (thin leaves), later on plants produce more assimilates, which are accumulated in the leaves. These results are supported by Tardieu et al. (1999) who found that SLA is not a constant parameter and varies among individual leaves, growth stages and even during the day.

### **6.1.3 Effects on transpiration**

In the hot wet season, only eight transpiration measurements were taken early in the season due to rain events preventing measurements and technical problems with the LCi. These problems were solved until end of November, which led to delayed beginning of measurements in the cold dry season. The differences between leaf levels were small for young plants and became larger with leaf age (as it was observed in the cold dry season). Since only young plants were measured, no differences between leaf levels were found (Figure 13).

In the cold season (Figure 14), transpiration rate differed between leaf levels in the flooded treatment, but not in the water-saving treatment. In the flooded treatment, leaf level of the youngest fully developed leaf showed the greatest transpiration rates. Transpiration decreased within the lower leaf levels. In the water-saving treatment, no significant differences between the leaf levels were found (Table 8). Since plants show a decreased leaf area under deficit irrigation, light interception is generally higher and more light arrives at the lower leaf levels. Furthermore, due to rat damage of the surrounding hills, remaining hills in the water-saving treatment were more exposed to sunlight, especially the lower leaf levels. Between treatments, only leaf level 1 differed significantly in transpiration rates. Since leaf level 1 is likewise exposed to sunlight in both treatments, it appears to be the appropriate leaf level for comparative transpiration measurements.

Considering the whole hill, differences between treatments were found for IR64 but not for IR4630 (Table 6). This contrasts with Davatgar et al. (2009), who posted that water-stress affected transpiration rates by closure of stomata and change in leaf morphology. In IR4630, lower transpiration rates of the higher leaf levels in the water-saving treatment compared to the flooded treatment were compensated by relatively high transpiration rates in the lower leaf levels. This was not the case for IR64. If this effect is caused by the different morphological characteristics of the varieties or attributed to different physiological traits, remains unclear and needs further investigation.

A depression of transpiration around noon (which was expected especially for plants in the water-saving treatment because it was observed by Turner et al. (1986) even for well watered plants) could not be observed for most of the plants and days. This finding fit to the results of Maruyama et al. (2008) who measured stomatal conductance and rarely observed a midday depression of photosynthesis. Dingkuhn et al. (1990) found a dependence of plant water deficits to the degree of afternoon depression, which was induced by partial stomatal closure. It can be concluded that plants in the water-saving treatment did not suffer on water deficits.

## **6.2 Using SLA for leaf area estimation**

This work shows that it is possible to estimate of leaf area until flag leaf appearance via SLA of the youngest fully developed leaf of the main tiller and leaf dry weight of the whole hill. Until flag leaf appearance, calculated and measured leaf area are closely related. After flag leaf appearance, significant differences were found (Figure 12). It can be concluded that the flag leaf is not suitable for leaf area estimations. In the cold

season, the differences between calculated and measured leaf area appear to be smaller compared to the hot season. Probably decreased tiller number in the colder environment led to a smaller tiller effect. Furthermore, the share of leaf level L4, which varies the most from the youngest fully developed leaf, in the whole plant is smaller in the cold season (Figure 7).

For further leaf area estimation, two methods can be recommended:

If many plants need to be assessed and little variation is acceptable, SLA of the youngest fully developed leaf of the main tiller can be used (Figure 12). Significant differences (Figure 12) between estimated leaf area using the youngest fully developed leaf and using the whole tiller occurred mainly after flag leaf appearance. After flag leaf appearance, the second youngest leaf is recommended to be taken.

For higher accuracy, leaf level 1 and 2 should be used. Primary, secondary and tertiary tillers should be included for a more precise estimation, since different tillers of the same tiller showed a large variance in terms of SLA. The younger a tiller, the larger is its SLA compared to the main tiller since SLA always starts large and gets smaller during leaf development (see Figure 9). A high tiller number leads to a low share of the main tiller on total leaf area. That means that taking only the main tiller for SLA measurements lead to underestimation of SLA.

### **6.3 Estimation of transpirational water loss on field level**

It is possible to estimate transpirational water loss on field level via individual leaf gas exchange measurements and leaf area. Field lysimeters are error prone and at the time of writing the data from the field lysimeters in the RISOCAS trial were not yet available. Thus, estimated transpiration was compared with ORYZA2000 calculations of transpiration. ORYZA2000 is a model, simulating growth, development, and water balance of rice under flooded or water-limited conditions (Wopereis et al. 1996b). Estimated cumulative transpirational water losses from leaf surfaces showed only small differences compared to transpiration simulated with ORYZA2000 (Figure 18).

The difference between estimated and simulated transpiration can be either explained with model deficiencies or methodical errors in the measurement. Possible errors are the exclusion of leaf level 0, consideration of leaf number 9-13 only or the unintended exclusion of a specific weather pattern, which could have changed the results.

Roel et al. (1999) calculated transpiration via subtraction of evapotranspiration and evaporation determined with lysimeters and cumulated transpiration over the cropping period. They found a cumulated transpiration of 150 mm at about 80 DAS in a most of

the time flooded field, which fits to the results estimated in this study. At 80 DAS, cumulated transpiration of 117 mm (IR4630) and 138 mm (IR64) were calculated for the flooded treatment in this study.

Estimating transpiration on field level via individual leaf transpiration measurements can save time and money. During field preparation, time consuming lysimeter installation can be omitted and during the growth period, labour-intensive data collection from lysimeters is not necessary. For precise measurements, lysimeters need to be installed in many replications and can be used in a few seasons only, due to corrosion of the material. Furthermore, typical problems with lysimeters, like disturbed soil or heating of the metal by solar radiation can be avoided. As shown here, individual leaf transpiration measurements need to be taken at some contrasting days only, which should be chosen carefully to cover the complete range of weather conditions. Constraints of this method are exhausting measurements during the whole day on the chosen measurement days, handling of a complex and sensitive measurement device, and the need of accurate measurement of the all leaf levels and precise leaf area measurement or estimation.

## 7 Conclusion and Outlook

This work was conducted to evaluate the effects of water-saving irrigation and climatic conditions on physiological parameters. Two varieties were assessed with regard to phyllochron and transpiration and five varieties with regard to leaf area and SLA. Leaf area, SLA and transpiration vary within seasons and treatments, lower values were observed in the cold season and in the water-saving treatment. Differences were found between varieties with regard to all three parameters. For leaf area estimation via SLA and leaf dry matter, the measurement of leaf level 1 and 2 of several tillers can be recommended. Leaf area calculation based on SLA of the youngest fully developed leaf of the main tiller usually underestimates leaf area of the entire hill. Transpiration rates are higher in the hot season. In the hot season, no differences between leaf levels were found. In the cold season, IR64 showed a significant lower transpiration under water-saving irrigation compared to the flooded treatment, whereas IR4630 maintained its transpiration under both treatments. Under flooded conditions transpiration rates decreased with leaf levels, whereas under water-saving conditions transpiration rates did not differ significantly for the leaf levels. Transpiration rates based on single leaf measurements can be extrapolated to estimate transpiration on plot level.

For a better understanding of physiological reactions to climatic conditions, measurements should be repeated in different seasons. Leaf level specific SLA should be determined for primary, secondary and tertiary tillers under consideration of adequate methods to estimate leaf area of the entire hill. For methodological validation of varietal differences in transpiration on field level and further evaluation of differences between cumulative transpiration calculated with ORYZA2000, transpiration measurements and lysimeter measurements, evapotranspiration and transpiration could be additionally measured with lysimeters.

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