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Crop Water Stress Management in the Tropics and Subtropics

Germination and early seedling partitioning of lowland rice in variable thermal conditions

Thesis prepared for the degree Master of Science

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I ABSTRACT

Rice production environments are quite diverse and often cropping calendar options are limited by either water availability or low temperature. Sustainable intensification of rice production systems requires flexibility in the choice of cultivar, sowing date, and management options. In transplanted systems, nurseries provide options to exploit early genotype x environment to the advantage of later development stages. In order to target nursery management to specific systems changes, it is important to know germination and early seedling vigor capacity of genotypes potentially subjected to new growing environments. Therefore, germination dynamics, mobilization efficiency of seed reserves, days to onset of photo-autotrophy and partitioning of early dry matter gains to the different organs was investigated under temperature regimes typical for tropical low altitude (28/20°C day/night) and tropical high altitude systems (20/12°C day/night) for six contrasting irrigated lowland rice genotypes. Germination dynamics was determined by calculating germination percentage, time taken to 50% of germinating seeds (T_{50}), mean germination time (MGT), and synchrony of germination (Z).

In a second experiment, rice was grown in wet loamy sand in climate chambers and destructively sampling of plant organs and remaining kernel was carried out after seedling emergence in certain time intervals.

While germination percentage did not differ significantly among the cultivars and temperature regimes, T_{50} and MGT was greatly extended when exposed to low temperatures. In *japonica* cultivars, mobilization of grain reserves during early growth of the seedling resulted in smaller respiration losses than in cold sensitive *indica* genotypes and thus a higher mobilization efficiency. In contrast, cold sensitive genotypes reached photo-autotrophy earlier at low temperature treatment but they took longer to compensate their initial kernel weight, resulting in a reduced early seedling vigor.

Keywords: low temperature, germination dynamics, *Oryza sativa*, management option, dry matter partitioning, reserve mobilization efficiency

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ABA	Abscisic acid
ANOVA	Analysis of variance
ASC	Asorbic acid
ATP	Adenosine triphosphate
°C	Degree Celsius
° Cd	Degree Celsius per day
DAS	Days after sowing
DD	Degree days
DW	Dry weight
GA_3	Gibberellin
GC I	Growth chamber I
GC II	Growth chamber II
GI	Germination index
h	hours
IRRI	Rice Research Institute
LAR	Leaf area ratio
LSD	Least significant differences
LTG	Low temperature germinability
M_ε	Mobilization efficiency
MGT	Mean germination time
mL	Milliliter
mm	Millimeter
N	Nitrogen
NARS	National Agriculture Research System
P	Probability value
RGR	Relative growth rate
R_l	Respiration losses
ROS	Reactive oxygen species
SLA	Specific leaf area
SLDW	Seedling dry weight
SRDP	Seed reserve depletion percentage
SRUE	Seed reserve mobilization efficiency
t	Ton

T_b	Base temperature
T_{max}	Daily maximum air temperature
T_{min}	Daily minimum air temperature
T_{50}	Time taken to 50% germination of seed
WMSR	Weight of mobilized seed reserves
%	Percentage

1 INTRODUCTION

1.1 *Oryza sativa* – "the daily food"

Rice (*Oryza sativa* L.) is an annual grass, which belongs to the family of the Poaceae (Chauhan et al., 2017). It is one of the earliest domesticated and one of the most important cereal in the world, a stable food source for over half of the world's population and is grown by more than half of the world's farmers (Fairhurst and Dobermann, 2002; Khush 2005). In some Asian languages, the word rice is synonymous with "daily food" which illustrates the great importance of rice as one of the major food sources in the world (Büchner, 1977).

Rice is grown on 11% of the world's cultivated land, with the highest rice production in China (210 million tons), followed by India (159 million tons), Indonesia (77 million tons), Bangladesh (53 million tons), Vietnam (43 million tons), Myanmar (26 million tons) and Thailand (25 million tons) (FAO, 2016). Of these 160 million hectares of rice land worldwide, 30% is subjected to salinity problems, 20% to drought and 10% to low temperatures at high latitudes and altitudes (Basuchaudhuri, 2014). Abiotic stress, especially with regard to temperature is a major problem for rice production worldwide due to the changing climate conditions (Singh et al., 2017).

Based on phylogenic studies, a distinction is made between two cultivated species, *Oryza sativa*, the Asian cultivated rice, which is grown all over the world and *Oryza glaberrima*, the African cultivated rice, which is grown on a small scale in West Africa. Furthermore, the cultivars are differentiated into two major groups *japonica*, which includes the *temperate japonica*, *tropical japonica* and *aromatic* subpopulations and *indica,* which comprises the *indica* and *aus* subpopulations (Garris et al., 2005). The *japonica* rice ecotype is mainly found in temperate regions with high altitude, whereas the *indica* rice ecotype is predominantly cultivated in subtropical regions with either low latitude or altitude. In addition, *japonica* and *indica* differ in their morphology such as plant height, agronomic features such as their persistence of seeds, as well as in their physical-biochemical characteristics like winter hardness (Lu et al., 2009).

1.2 Production systems of rice cultures

Rice is grown under very different ecological conditions. Five major ecosystems are generally classified as followed: (1) Irrigated lowland, (2) Rainfed lowland, (3) Coastal wetlands, (4) Deepwater and (5) Upland rice (Basuchaudhuri, 2014). Transplanting is the most widely used method in rice production and 77% of rice was transplanted globally in 2007 (Rao et al., 2007).

However, this conventional rice system demands large amounts of water, energy input and labour and thus, is no longer suitable for sustainable development. Direct-seeded rice seems therefore to be a promising alternative option for rice production with its advantages of saving water and labour (Liu et al., 2015; Kumar and Ladha, 2011; Hyun et al., 2017).

1.3 Future challenges in rice cultivation

Rice production has to increase by at least 25% until 2030 to keep pace with the predicted population growth (Li et al., 2014).

Due to the ongoing climate change as well as the need to cultivate additional land for the growing demand of food supply, rice production has increasingly extended into higher altitudes. As a consequence, a slow and irregular germination as well as a poor crop establishment induced by predominantly lower temperatures in higher altitudes systems causes yield reduction and impedes the method of direct-seeded rice in hilly areas (Rao et al., 2007).

Furthermore, most of the seasons are limited by temperature so rice cultivation is only possible in a short period of time in the year, thus a rapid early development of rice seedlings is a prerequisite for a successful rice cultivation in high altitude systems (Sasaki, 1983).

In addition to the expansion of production area into higher altitudes, further increases must also be met by enhancing the yields through improved crop management. For that purpose, a better understanding of growth and development processes of rice seedlings is necessary for the selection of genotypes which are well suited to specific management strategies and/or environmental conditions.

1.4 Objectives

The aim of this study was therefore to gain an understanding of early growth processes of rice seedlings under various temperature regimes. In particular, the objective was to clarify the effects of low temperature on the germination dynamics of different rice cultivars and furthermore, to quantify the fraction mobilized for growth, identify the onset of the autotrophic phase, as well as the corresponding respiration losses. For that purpose, various germination indices were calculated and the partitioning of dry matter to shoots, roots and leaves, as well as the kernel dry weight loss were determined. Six different cultivars were chosen to detect if these processes are affected by cultivar and/or temperatures.

2 LITERATURE REVIEW

2.1 Low temperature affecting rice production

Rice is principally a crop which is native to tropical, sub-tropical and temperate regions, yet it is also cultivated in high latitude or high altitude regions of Australia, China, Japan, Nepal, Russia, Korea and other rice growing countries (Hyun et al., 2017; Jena et al., 2012; Farrell et al., 2004). For example, during cold snaps in Australia, cold stress causes a 40% yield loss due to cold-induced sterility (Naidu et al., 2005). In India, rice grown in cooler regions accounting to 2.3% of the total rice producing area. The average yield of this rice is about 1.1 t h^{-1} as compared to the average of the national yield of 9.1 t h^{-1} (Peyman and Hashem, 2010). Due to its origin and the fact, that it is a tropical C_3 crop, rice is more sensitive to low temperatures than other cereal crops such as wheat (*Triticum aestivum* L.) or barley (*Hordeum vulgare* L.). Optimum temperatures for rice germination and seedling growth is estimated to range from 25°C to 35°C. While temperatures below 10°C can result in a complete failure of germination (Yoshida, 1981b). Cold stress occurs when temperatures fall below 17°C, causing poor germination, extended germination period, slow seedling growth, leaf discolouration and mortality (Shakiba et al., 2017). However, the critical temperature for rice growth can be different and depends on cultivar and the time the plants are exposed to low temperatures (Yoshida, 1973). A general overview of critical temperature ranges for each growth stages is provided by Table 1.

In summary, low temperatures or chilling stress ultimately reduces yield and increases the competition with weeds especially in direct-seeded rice systems. Therefore, vigorous germination and rapid seedling growth is the key for the establishment of stable and profitable rice plants.

Table 1
Critical temperature ranges for various growth stages of rice (Singh et al., 2017)

Growth stage	Critical temperature in °C		
	Low	Optimum	High
Germination	16-19	18-40	45
Seedling emergence and establishment	12-35	15-30	35
Rooting	16	25-28	35
Leaf elongation	7-12	31	45
Tillering	9-16	25-31	33
Initiation of panicle primordia	15	-	-
Panicle differentiation	15-20	-	30
Anthesis	22	30-33	35-36
Ripening	12-18	20-29	>30

2.2 Low temperature affecting plant development

2.2.1 Germination

Germination is the growth of an embryonic plant contained within the seed. It starts with the uptake of water (imbibition) and ends with the appearance of the radicle or coleoptile through the seed coat (Ichie et al., 2001). The uptake of water by dry seeds is triphasic (Fig. 1). The initial water influx (Phase I) comes due to the low water potential of the cell walls and the storage components in the seed. The influx of water in this Phase causes transient structural perturbations to the membranes, with the consequence of an ion and low-molecular weight metabolites efflux from the seeds. The seeds then rapidly become hydrated, which results in a plateau (Phase II). Phase III is characterized by a further increase in water uptake, which occurs only after germination is completed and the embryo grows into a seedling.

Hydrated seeds rapidly continue with their metabolic activities like respiration, which occurs already within minutes of the start of imbibition, as well as the activation of several enzymes to produce ATP in the mitochondria, located in the cell tissue of the seeds, to support the metabolism for several hours after imbibition.

The germination is completed when the radicle or the coleoptile permeates through the seed coat. This is the result of cell extension, which is a turgor driven process (Bewley, 2001).

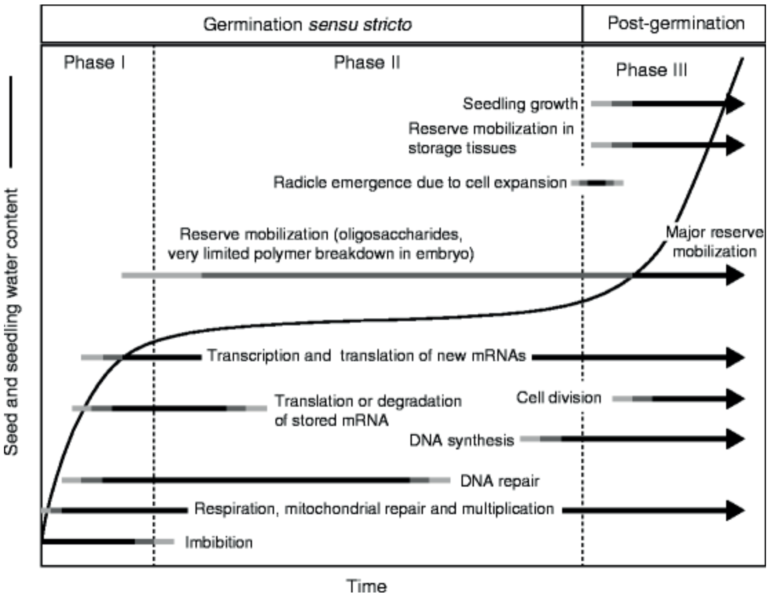

Figure 1 The time course of events of seed germination and postgerminative seedling growth (based on Bewley, 1997)

The entire germination process is greatly affected by temperature. Low temperature reduces seed germination rate and also slows down the entire germination process. This starts from imbibition, activation and subsequent manifestation (Basuchaudhuri, 2014).

In Phase I, low temperature slows down the diffusion of water into the seed. Then, the incomplete plasma membrane of the dry seed causes an increased escape of solutes. As a result, the stress hormone abscisic acid (ABA) increases, this leads to a decrease of reactive oxygen species (ROS) in imbibed rice seeds and induces therefore an inhibition of the production of ascorbic acid (ASC) and gibberellin (GA_3), as well as an overall unbalanced metabolic and enzymatic activity (Fig. 2) (Zhang et al., 2012). Those irregular metabolic activities can also affect cell extension and cell division (Lyons, 1973).

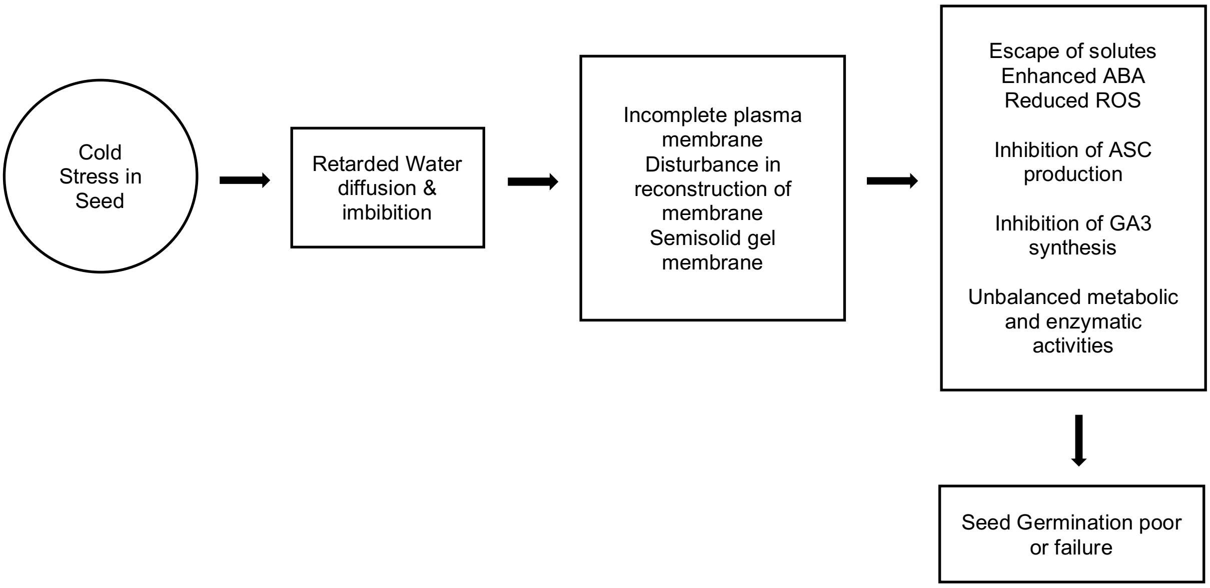

Figure 2 Mechanism of cold effects on Germination (Basuchaudhuri, 2014)

2.2.2 Early seedling stages

During the early seedling stages the occurrence of low temperature has a strong impact on growth, survival and reproduction. If seedlings ever survive, it can lead to nonuniform crop maturation and eventually to a reduced productivity (Singh et al., 2017).

Visual symptoms such as wilting and yellowing of the leaves at seedling stage can be one obvious sign for cold intolerance caused by changes in physiological activities. At cooler temperatures, the rate of transpiration and ion leakage increases, while the photosynthesis as well as the carbohydrate metabolism decreases (Su et al., 2010; Gagoi and Baruah, 2000). The reduction in photosynthetic activities is induced by a starch build-up in the chloroplasts, which inhibit the photosynthetic enzymes (Sonoike, 1999).

In general, the *japonica*-type cultivars are known to be less susceptible to cold stress during early seedling growth than *indica* cultivars (Pillai and Akiyama, 2004). Hence, cold tolerance at the early growth stage can be one of the characteristics for distinguishing the two subspecies of cultivated rice (Baruah et al., 2008). Tolerant seedlings, when exposed to low temperatures, have partially adapted morphologically by reducing their leaf area ratio (LAR), specific leaf area (SLA) and relative growth rate (RGR). In addition, they increase their leaf thickness to protect the photosynthetic machinery (Verheul et al., 2009). Another indicator for tolerant seedlings is a high respiratory homeostasis. In other words, some rice cultivars can maintain similar respiratory rates (measured at the growth temperature), even when grown at different temperatures (Kuk et al., 2003).

2.3 Rice germination and seedling growth under various temperatures regimes

Low temperature germinability (LTG) is an essential trait of rice cultivars used in direct-seeded cultures especially in higher altitude systems. Nishiyama (1977) reviewed the effects of temperature on germination and vegetative growth of rice plants. He reported, that *indica* varieties show higher minimum temperatures of germination than *japonica* varieties. Furthermore, he could demonstrate in one of his experiments that germination ability and root elongation clearly declined below the critical temperature of 17°C.

Sasaki (1983) could verify, that there is a significant positive correlation between LTG, which was defined as ratio of germination percentage to average number of days required for germination and seedling elongation at an early stage of growth, rooting ability, root development and dry weight of seedlings at low temperatures. Similar results were recorded in the experiment of Sahu et al. (2014) who measured root and shoot length of 50 rice genotypes exposed to 15°C and 28°C for two weeks. The results revealed furthermore, that there is a positive correlation between root length and germination percentage. He also considered rice genotypes with a germination percentage between 40% and 80% at low temperatures as tolerant. Nahar et al. (2009) studied the differences of 244 genotypes with contrasting growth durations. His results indicated that tolerant genotypes having a short growth duration might have the ability to withstand low temperatures while genotypes with long growth durations on the other hand seem to have different avoidance strategies.

Ye et al. (2009) investigated the cold tolerance of 17 different rice varieties at different growth stages. Low temperature germinability got estimated by calculating the germination percentage, the mean germination time (MGT) as well as the germination index (GI) of rice seeds germinating at 28 and 15°C. At 28°C most of the seeds germinated within five days and the majority of the varieties showed high germination percentages while at 15°C germination was slower, with no germination until the third day of the cold treatment and a reduction in germination percentage for most of the varieties. He came to the conclusion, that germination rate and mean germination time are not correlated because some varieties with high germination percentage germinated very slowly. Therefore, he recommended to use the germination index (GI) which combines the germination rate and time to evaluate cold tolerance at germination stage. For an even more accurately determination of cold tolerance, GI should also be used in a combination with germination rate and mean germination time.

With respect to seedling growth at low temperature, Ye et al. (2009) also revealed that the number of leaves, leaf elongation rate, number of tillers, plant height, and shoot dry weight were highly correlated. Shoot dry weight was therefore an effective indicator for the number of leaves, leaf elongation rate, number of tillers as well as plant height.

The effect of temperature on decomposition of reserves during germination and early growth of rice plants was studies by Zakaria et al. (2001). For that purpose, he exposed two different rice cultivars to low temperatures of 17°C, ambient temperatures of 16-24°C and high temperatures of 38°C and examined the endosperm cells with a transmission electron microscope. At temperatures of 17°C the structure of endosperm cells did not change during the first days of sowing (DAS) and starch granules were still strictly enveloped in the amyloplast membrane, while at high temperatures of 38°C the structure of the endosperm cells changed already at 1 DAS. Only at 5 DAS a groove-like structure appeared on the surface of the amyloplasts at 17°C. At 10 DAS the amyloplasts envelope were dissolved and the starch granules were separated from each other. At 15 DAS the starch granules increasingly decomposed and therefore got smaller, except of some starch granules at the distal end of the seed as well as some protein bodies among the amyloplasts. which did not decompose until that time. At temperatures of 16-24°C and 38°C the starch granules had increasingly decomposed at 10 DAS and were almost completely decomposed at 15 DAS at 38°C and at 20 DAS at 16-24°C. Not only the speed of decomposition varied between the different temperatures, also the structure of the starch granules varied between 17°C and the other two temperature treatments, which might be the result of a low activity or insufficient number of degradation enzymes.

They furthermore found out, that at low temperatures of 17°C the seminal roots emerged at 5-7 DAS, while at constant temperatures of 32°C the seminal roots already emerged at about 32 hours after imbibition. Those results indicate, that the decomposition of starch granules is one important key factor for germination.

2.4 Reserve mobilization and dry matter partitioning

Rice seedlings start as heterotrophic, fully depending on nutrient mobilization from the endosperm. Subsequently they pass through a transition phase when photosynthesis starts but the reserve mobilization from the endosperm still continues until the seedlings finally depend entirely on photosynthesis and reach the photoautotrophic phase (Salam et al., 1997). The knowledge about the duration of each phase is a necessary tool to improve rice cultivation under certain management, cropping calendars and/or environmental condition. In direct-seeded cultivation systems for example a rapid seedling growth is important for an improved ability to tolerate weeds (Asch et al., 1999; Namuco et al., 2009). Also, the timing of the first urea application which is a highly volatile fertilizer could be applied to the plant stage, when the seedling is able to utilize urea. This would reduce N losses, as well as soil and water contamination with nitrates (Salam, 1997).

Yoshida (1973) studied the effect of different temperatures, with mean temperatures ranging from 22 to 31°C on early seedling growth of the rice cultivar IR 8 in a controlled environment. For this purpose, he sampled entire rice seedlings growing at different temperatures every week for a three weeks period and separated them into shoots, roots and kernels. He found out, that in the first week of growth, the photosynthesis contributed for only 30% or less to growth and therefore the seed reserve supported the rest. During the second week, photosynthesis was responsible for 84% of growth and during the third week, growth was totally supported by photosynthesis. This result was similar for all temperatures.

However, temperature had a great effect on the growth rate, which increased gradually with increased temperature, especially in the first week. Yoshida therefore concluded, that the enzymatic breakdown of the seed reserves must be greatly affected by temperature, because the seed reserves are responsible for more than 70% of growth during the first week of growth.

Asch et al. (1999) studied the kinetics of reserve mobilization and dry matter partitioning among organs of five different rice cultivars (*O. glaberrima* landrace vs. *O sativa* japonica types) at a temperature of 24.6°C. During the first days, the seedling growth was fully heterotrophic and the total dry matter decreased by about 10% due to respiration costs. This phase was followed by the photo-autotrophic phase, which was characterized by exponential growth pattern. The kernel dry weight decreased linearly until 10 to 12 days and then remained constant at about 22 to 26% of the initial kernel weight. The different cultivars did not differ significantly in the amount of mobilized kernel dry weight but did so in the relative rates of mobilization. The general dry matter distribution of the seedlings was very similar in all cultivars. Dry weight growth was smallest for roots and intermediate for shoots. The different cultivars also showed similar patterns for DW ratios between the roots and the total seedling, but they differed significantly regarding the DW ratio between the leaf blades and the total seedling. In addition, root growth declined between the onset of autotrophy until the reserves were completely exhausted. This period coincides with the development of the photosynthetic apparatus. Even though no big differences between the cultivars were observed. With these results, Asch et al. (1999) were able to demonstrate that a superior early vigor of the *O. glaberrima* landrace at an early seedling stage is due to a rapid onset of the autotrophic growth phase in connection with a fast reserve mobilization, a high partitioning coefficient of the leaves, as well as a large specific leaf area.

Salam et al. (1997) set up an experiment to identify preautotrophic phases, to quantify seed dry matter mobilization and utilization, as well as to characterize dry matter partitioning during early growth of two popular rice genotypes from Bangladesh (BR 11 and BR 14). For that purpose, he sampled entire rice plants growing in soil, daily over a period of 27 days after emergence and separated them into root, culm and leaf. The changes in dry weights of seeds and seedlings were used to determine the duration of the heterotrophic, transition and autotrophic phases. The rice seedlings became photoautotroph by 321 thermal units (DD, °Cd)[1].

His results coincide with those of Yoshida (1973) when converting his output into thermal units. The duration of 321 thermal units appeared to be independent of cultivar and seed size. Dry matter partitioning during the autotrophic phase showed an almost constant culm-to-leaf partitioning of shoot dry matter, while root-to-shoot partitioning decreased continuously up to 148 thermal units.

An investigation of seed reserve utilization and seedling growth of wheat as affected by drought and salinity was carried out by Soltani et al. (2006). The aim of this study was the identification of the sensitive seedling growth components defining the heterotrophic phase in response to two abiotic stresses. Soltani et al. (2006) defined this growth phase as product of the weight of mobilized seed reserve and the conversion efficiency of utilized seed reserves to seedling tissue. The results of this study indicated that the decline in seedlings dry weight in response to drought and salinity was a consequence of a decline in the weight of mobilized/utilized seed reserves and not the conversion efficiency to the seedlings tissue.

Physiological responses of a cold-sensitive and a cold tolerant rice cultivar got studied by Aghaee et al. (2011). Temperature treatment of 15/10°C day/night compared to 29/22°C day/night showed, that growth was negatively correlated with the cold treatment and seedlings growing at conditions of 29/22°C day/night had significantly higher dry weights compared to the cold treated ones in both cultivars. However, the cold-tolerant cultivar showed a decreased shoot/root ratio mainly due to a higher root dry matter. Therefore, it seems likely, that a high root biomass is correlated with cold tolerance and a reduction in shoot biomass and plant height could be an avoidance strategy.

Early seedling vigor is a desirable trait for crop establishment especially in direct-seeded cultivation systems in unfavorable growing conditions. Huang et al. (2017) investigated

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[1] $DD = \sum ((T_{max}+T_{min})/2-T_b)$
T_{max} = daily maximum air temperatur, T_{min} = daily minimum air temperature; T_b= base temperature of 9°C).

therefore the morphological traits associated with early vigor of two contrasting rice cultivars. His results demonstrated, that the cultivar with a superior early vigor showed a higher germination percentage, shoot biomass and leaf area, which therefore improves the photosynthesis. In addition, this cultivar allocated more dry matter to the shoot but less to the roots during the early autotrophic growth phase than the cultivar with average early vigor. This led to the assumption, that a higher root biomass would not absolutely promote shoot growth due to consuming assimilates produced by the shoot for increasing root growth.

3 MATERIALS AND METHODS

3.1 Plant material and treatments

The experiments were conducted in two growth chambers (Percival Scientific Inc., Perry, IA, USA) at the Hans-Ruthenberg Institute of the University of Hohenheim between the 28th of November 2017 and 24th of April 2018.

Seeds of six different rice cultivars (Chomrong, Faro 35, IR 64, X265, X jigna, and Yun Keng) provided by the institute for crop production and agroecology in the Tropics and Subtropics of the University of Hohenheim, were used in all experiments.

Chomrong is a traditional, temperate *japonica* rice ecotype from Nepal, a short-duration cultivar that is known to be cold tolerant and adapted to high altitudes (Julia and Dingkuhn, 2012). The cultivar Faro 35 also known as ITA 212, is an improved, high yielding, lowland rice variety, which got released in Nigeria in 1986 (Imolehin and Wada, 2000). IR 64, an improved lowland cultivar was developed by the International Rice Research Institute (IRRI) in the Philippines and is widely grown in South and Southeast Asia (Deshmukh et al., 2017).

The cultivar X 256, also called Mailaka is a variety also bred by the IRRI and selected by the National Agriculture Research System (NARS) for the rainfed lowland growing areas of Madagascar (Walker and Alwang, 2015). X jigna, a cold tolerant variety is mostly grown in the mid-highlands of Ethiopia (Zenna et al., 2010), and Yun Keng, a short-grain *japonica* rice, is known for its cold tolerance and therefore it is mostly grown during the cold season in Rwanda (Zenna et al., 2010). Further details about the cultivars are represented in Table 2.

Table 2
Characterization of the different rice cultivars used in all experiments

Cultivar	Species	Genetic group	Ecotype	Specific traits	Country of origin	Reference
Chom-rong	*O. sativa*	temperate japonica	traditional upland	Cold tolerant	Nepal	Julia and Dingkuhn, 2012
Faro 35	*O. sativa*	indica	irrigated lowland	Salinity tolerant, high yielding	Nigeria	Evenson and Gollin, 2003; Sparks, 2005
IR 64	*O. sativa*	indica	improved lowland	high yielding, semi-dwarf variety	Philippines	Datta et al., 2006
X 265	*O. sativa*	[1]	improved lowland	drought tolerant, cold tolerant, short-cycle	Madagascar	ASARECA, 2016
X jigna	*O. sativa*	temperate japonica	highland	Cold tolerant	Ethiopia	Ndour et al., 2016
Yun Keng	*O. sativa*	temperate japonica	lowland	Cold tolerant	Rwanda	Zenna et al., 2010; Page, 2015

[1] no clear information about the genetic group of X 265 was found

The effects of two various temperature regimes on germination dynamics, dry matter partitioning and the kinetics of reserve mobilization of the six different cultivars were investigated. Two growth chambers (GC I and GC II) were set for optimal conditions at 28/20°C day/night and for low temperature conditions at 20/12°C day/night. These temperatures were chosen because they are typical for tropical low altitude (28/20°C day/night) and tropical high altitude systems (20/12°C day/night) and therefore simulate two exemplary environments for rice production. In addition, many studies showed, that the physiological critical temperature for early seedling growth of rice is approximately at 17°C and therefore a temperature effect was to be expected (Ogiwara and Terashima, 2001). The photoperiod was 12/12 h light/dark in each growth chamber for seedling growth. The temperature increased and decreased slowly over a period of two hours (Fig.3).

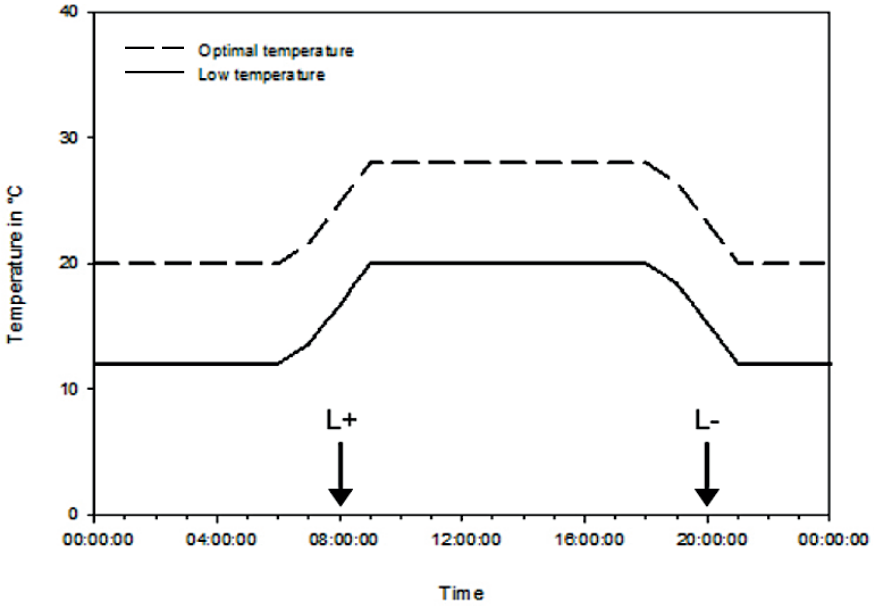


Figure 3 Time courses of temperatures in growth chambers. Optimal conditions = 28/20 °C day/night; low temperature = 20/12 °C day/night; with a photoperiod of 12/12 h day/night; L+ = lights on; L- = lights off.

Figure 4 Seed shapes of different rice cultivars used in all experiments. Top, from left to right: IR 64, X jigna, X 265; below, from left to right: Chomrong, Faro 35, Yun Keng.

3.2 Experiment I: Germination dynamics in variable thermal regimes

3.2.1 Preparation: Kernel dry weight distribution

To ensure an equal initial kernel dry weight for all plants, a 20 g seed sample of each cultivar was taken to determine kernel weight distribution (Fig. 5). The seeds were ranked by weight in five categories: < 20 g; 20-25 g; 25-30 g; 35-40 g; > 40 g. Seeds of each cultivar, which were similar in size and appeared flawless, were then selected from the category of the calculated mean (Tab. 6) and used in all experiments.

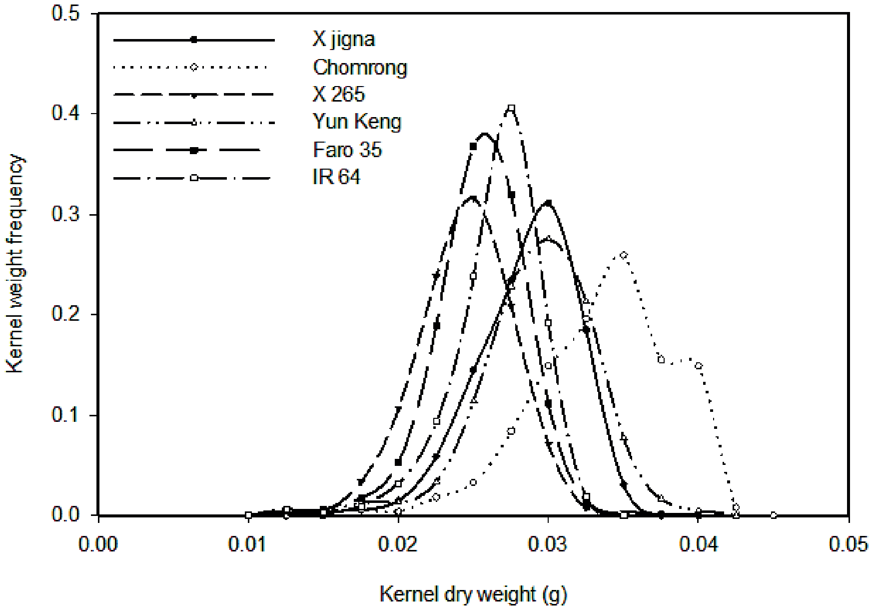


Figure 5 Frequency distribution of the kernel dry weight for 20 g samples of each cultivar.

3.2.2 Experimental set up

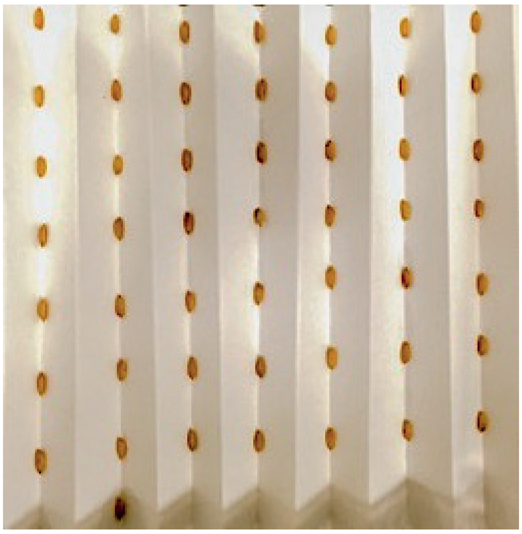


Figure 6 Seed arrangement in PET Plate using the example of X 265.

50 seeds of each cultivar were placed in PET plates of 19.5 x 19.5 cm, containing filter paper (Macherey-Nagel, MN 818, Düren, Germany), moistened with 15 mL of distilled water. The filter paper was folded like a hand-held fan to avoid possible slipping (Fig. 6). The seeds were spaced 2.5 x 2.5 cm in the plates, which then, were placed in a completely randomized design in the respective growth chamber. The lids of the PET plates were placed in such a way that aeration was ensured. The experiment was replicated two more times with 20

seeds per cultivar. The replications were performed in succession, always changing the two available growth chambers. Therefore, the experimental design layout was considered to be a split plot arrangement with main plots being organized in a row-column design with rows dedicated to replications and columns to growth chambers. The different cultivars were assigned to the subplots within each main plot (Fig. 7).

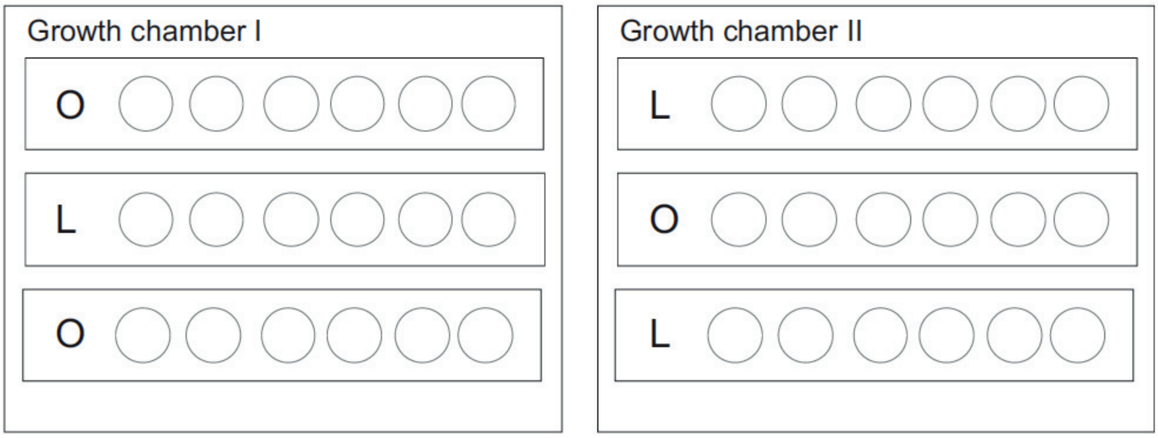


Figure 7 Schematic representation of the germination experiment. O = optimal conditions of 28/20 °C day/night; L = low temperature conditions of 20/12 °C day/night; outer square = climate chamber; inner square = replications, performed in succession; circles = cultivars.

Figure 8 Germinated seeds of X 265 after 6 days at 28/20 °C day/night.

From each PET plate the number of germinated seeds were recorded every 24 h over a period of seven days considering seeds germinating at 28/20°C day/night and over a period of 20 days for the seeds germinating at 20/12°C day/night. To keep the filter paper moist, 10 mL of distilled water was added into each plate containing the seeds germinating at 28/20°C day/night daily and 5 mL into each plate containing the seeds germinating at 20/12°C day/night every second day. The seeds were considered germinated, when the radicle or coleoptile has reached a length of approximately ≥ 1 mm (Hyun et al., 2017). Due to fungal infestation of the cultivars Yun Keng, X jigna and Faro 35 at low temperature treatment, the germination test was repeated after seed surface sterilization. Therefore, seeds were soaked in common laundry bleach (Dan Klorix, Colgate-Palmolive, Germany) containing 2.8% sodium hypochlorite for 30 minutes. The seeds were then rinsed thoroughly, with constant agitation, in four changes of 100 mL of sterile Millipore water (Ella et al., 2011; Chun et al., 1997). Subsequently, the seeds were placed on moist filter paper and the further procedure was carried out as described earlier.

3.2.3 Data collection

To obtain information about the entire germination process, germination percentage (G%), mean germination time (MGT), time taken to 50% germination of seeds (T_{50}) as well as germination synchrony (Z) are important aspects in consideration of the germinability of seeds and were accordingly often used in similar studies (Ranal and De Santana, 2006; Ranal et al., 2009). Therefore, these parameters were also chosen for evaluation in the present study.

Time taken to 50% germination of seeds (T_{50}) was calculated according to the formulae of Coolbear et al. (1984) and Farooq et al. (2005):

$$T_{50} = t_i + [(N / 2 - n_i)\ (t_i - t_j)] / n_i - n_j \qquad (1)$$

Where N is the final number of germinated seeds, n_i and n_j are the cumulative number of seeds germinated by adjacent counts at times t_i and t_j when $n_i < N/2 < n_j$. This mathematical expression of germination time focuses not only on the slower and faster seeds but takes the central tendency into account. Since the frequency of distribution of germination of all cultivars and treatments were symmetric, this formula was appropriate.

Mean germination time (MGT) was used to calculate the weighted mean of germination time. It therefore informs about the average length of time required for the maximum of germination of a seed lot (Czabator, 1962) and was calculated according to the equation of Ellis and Roberts (1980):

$$MGT = \sum n_i t_i / \sum n_i \qquad (2)$$

Where n_i is the number of germinated seeds on day t_i and t_i are the days counted from the start of the experiment.

The Z index describes the synchrony of one seed germinating with another seed, which is included in the same replication of one treatment. It was calculated as also proposed by Ranal and De Santana (2006):

$$Z = \sum C_{ni,2} / N \qquad (3)$$
$$\text{with } C_{ni,2} = n_i (n_i - 1) / 2$$
$$N = \sum n_i (\sum n_i - 1) / 2$$

$C_{ni,2}$ is the combination of the seeds germinated in time *i,* two by two and n_i is the number of seeds germinated at time *i.* If Z = 1 the germination of all seeds took place at the same time, if Z = O at least two seeds germinated, one at each time.

3.2.4 Experiment I: Dry matter partitioning

Ten randomly selected seedlings of each cultivar and temperature treatment grown on filter paper were divided into roots, shoots and remaining kernels, as soon as the shoots of the seedlings reached a height of 2 cm.

The separation was done carefully by holding the remaining kernel with tweezers and then using a pair of sharp scissors to remove shoot and root.

The shoots, roots and remaining kernels were individually packed in labeled, small papier bags for each seedling and oven-dried at 70°C for 72 h (Salam, 1997) to subsequently determine the dry weights with a precision balance (Precisa XB 220A; Precisa, Gravimetrics AG, Dietikon, Switzerland). The total seedling dry weight and root/shoot ratio was calculated. In addition, the weight of mobilized seed reserves (WMSR) was calculated as dry weight of the initial seed minus the weight of the remaining seed. The conversion efficiency of seed reserves into plant tissue (SRUE) got estimated by dividing the total seedling dry weight (SLDW) through the weight of the mobilized seed reserves. The ratio of utilized seed reserve to initial seed dry weight was considered as seed reserve deletion percentage (SRDP) (Soltani et al., 2006).

3.3 Experiment II: Reserve mobilization and dry matter partitioning in time course

3.3.1 Experimental set up

To estimate reserve mobilization efficiency and dry matter partitioning in the course of time, seeds were sown in multipot plates (Baumschulplatten, QT 35T; Burger GmbH, Renningen-Malmsheim, Germany) with the dimension 28.0 x 36.0 x 11.5 cm. Within the multipot plate each pot had the dimension 5.0 x 5.0 x 11.5 cm, which ensured a sufficient depth for root growth and avoided root entanglement due to the separation of each plant. The multipot plates had space for 35 plants and one plate was used for each cultivar. Therefore, the experiment had five replications (one seedling each) and the seedlings were harvested every second day over a period of 14 days after seedling emergence for plants growing at 28/20°C day/night.

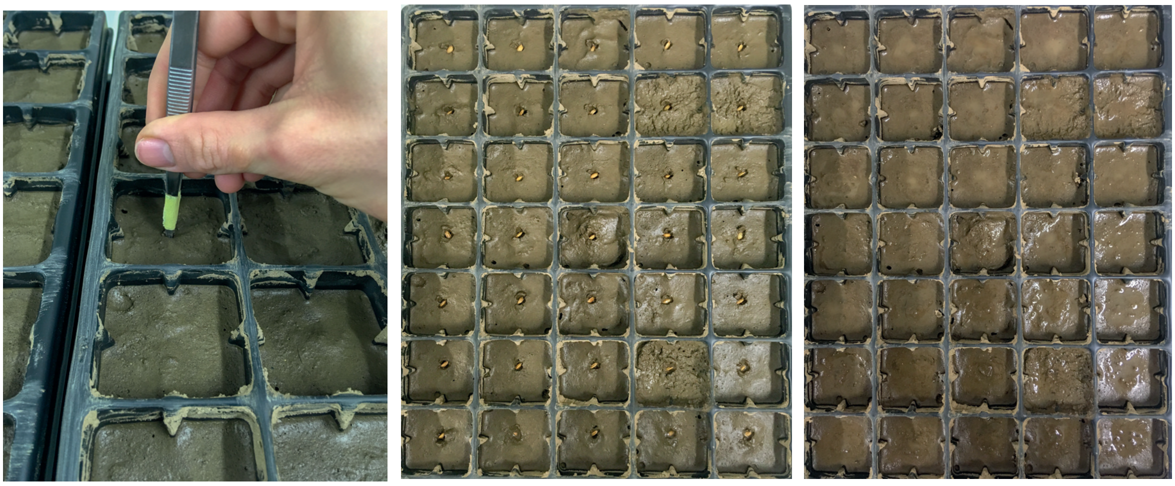


Figure 9 Seed sowing procedure. Left: Sowing the seeds in 0.5 cm depth by using a pair of tweezers; middle: seeds were places in such a way as to face the embryo to the horizontal direction; right: covered seeds in multipot plate.

Since seedlings exposed to low temperatures had slower growth rates, sampling took place every three days until the total dry matter increased, henceforward sampling was carried out every five days to ensure reaching the final kernel weight at the end of the experiment.

The selected seeds were soaked in distilled water for 24 h at room temperature before sowing (Ye et al., 2009). The soil was a finely sifted (2 mm) loamy sand. Each multipot plate contained about 3/2 parts of soil to 1/3 parts of water (6000 g soil, 3000 g water), which got well mixed to obtain a muddy texture. Two multipot plates were put together in a plastic planter with the dimension 59.0 x 39.0 x 6.0 cm. The soil-filled pots were then put in the growth chamber 72 h prior to sowing, in order to bring the soil to the corresponding temperature in the growth chamber. Seeds were sown in 0.5 cm depth, this was done by using a pair of tweezers with a corresponding mark at 0.5 cm (Fig. 9). Seeds were placed in such a way as to face the embryo to the horizontal direction (Ogiwara and Terashima, 2001) (Fig. 9).

The irrigation of the plants was simply carried out by adding water to the planter. Every 24 h it was ensured that they were half full of water. Each pot in the planter, as well as the planter itself got rearranged and rotated in the growth chamber every 24 h.

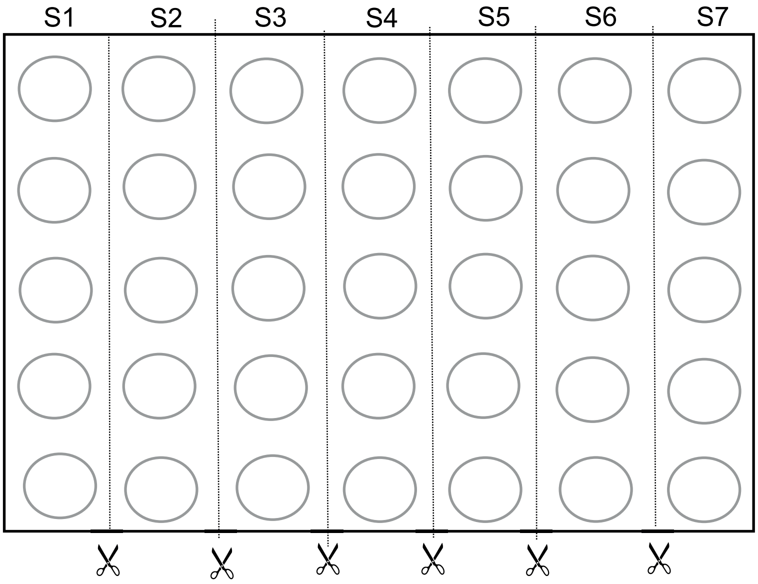


Figure 10 Schematic representation of one multipot plate (one plate was used for one cultivar); circles = seedlings; S1-S7 = Sampling dates 1-7, chosen randomly.

3.3.2 Sampling method and data collection

One entire seedling for each replication was sampled every second day after seedling emergence for plants growing at optimal conditions of 28/20°C day/night. Since seedlings of the different cultivars showed different times of emergence, the first date of seedling sampling was chosen individually (Tab. 3).

Table 3
Individual sampling dates of the cultivars exposed to 28/20 °C day/night

Cultivar	Sampling dates (DAS)[1]
Chomrong	8, 10, 12, 14, 16, 18, 20
Faro 35	[2]
IR 64	9, 11, 13, 15, 17, 19, 21
X 265	8, 10, 12, 14, 16, 18, 20
X jigna	8, 10, 12, 14, 16, 18, 20
Yun Keng	6, 8, 10, 12, 14 16, 18

[1]DAS = Days after sowing; [2] no emerge took place during the entire time of the experiment

Due to the slower growth rate, seedlings exposed to low temperature of 20/12°C day/night were sampled every third day until the total dry matter (roots, culm, leaf blades and remaining kernel) increased. Henceforward, sampling took place every five days, to ensure reaching the final kernel weight at the last day of sampling (Table 4).

Table 4
Individual sampling dates of the cultivars exposed to 20/12 °C day/night

Cultivar	Sampling dates (DAS)[1]
Chomrong	18, 21, 24, 27, 32, 37, 42
Faro 35	[2]
IR 64	18, 21, 24, 27, 32, 37, 42
X 265	18, 21, 24, 27, 32, 37, 42
X jigna	14, 17, 20, 23, 28, 33, 38
Yun Keng	15, 18, 21, 24, 27, 32, 37

[1]DAS = Days after sowing; [2] no emerge took place during the entire time of the experiment

Figure 11 Method used to obtain intact seedlings from the multipot plate. Left: sampling 2 (10 DAS) of X jigna seedlings exposed to 28/20 °C day/night; right: sampling 7 (38 DAS) of X jigna seedlings exposed to 20/12 °C day/night.

In order to sample entire, intact seedlings, one row of five pots of the multipot plates got cut off, as well as the front wall of the row so the entire seedling together with the soil could be taken out laterally and the excess soil could simply be separated from the seedlings under water (Fig. 11 and Fig. 12). The seedlings were soaked in moderately warm water for 30 minutes and then rinsed off in combination with gentle rubbing to remove the remaining, firmly adhering soil from roots and kernels.

The sampled seedlings were divided into roots, shoots (culm and leaf blades) and remaining kernel (Fig. 13) as described earlier, then packed in small, labelled paper bags and oven-dried at 70°C for 72 h. Subsequently, the kernel dry matter was determined by using a precision balance (Precisa XB 220A; Precisa, Gravimetrics AG, Dietikon, Switzerland), early root and shoot dry matter was determined by using a micro balance (Mettler Toldeo XS3DU; Mettler Toldeo, Gießen, Germany)

Figure 12 Method used to obtain intact seedlings from multipot plate. Left: seedling in soil after taken out from the multipot plate laterally; middle: seedling after rinsing off the excess soil; right: seedling after soaking in moderately warm water for 30 minutes.

Figure 13 Seedlings before and after destruction into shoot, root and remaining kernel. From left to right: 5 repetitions of X jigna seedlings at sampling date 6 (18 DAS) when exposed to 28/20 °C day/night; X 265 seedling at sampling date 4 (27 DAS) when exposed to 20/12 °C day/night before (left) and after destruction into the plant organs (right); Chomrong seedling at sampling date 4 (27 DAS) when exposed to 20/12 °C

The kernel dry weight loss was calculated according to the formulae of Asch et al. (1999):

$$\text{Kernel DW loss} = 1 - \text{final kernel weight} / \text{initial kernel weight} \tag{4}$$

To determine the days to onset of photo-autotrophy, which is characterized by the time when photosynthesis fully contributes to the growth of the seedlings, and to calculate the mobilization efficiency for each cultivar, a linear regression analyses according to Asch et al. (1999) was performed.

Based on two linear regression lines, one describing the kernel dry weight reduction (Regression I), the other one the total dry matter decrease due to respiration losses (Regression

II), and a regression curve which was fitted to the total dry matter (kernel, shoot and root) development, the respective parameters were calculated.

The onset of the autotrophic growth phase was determined as the culmination point ($x = 0$) of the regression of the total dry matter development, which usually increases after the plant enters the autotrophic growth phase.

The point where the seedlings photosynthesis fully contributes to the plant growth is given when all reserves have been mobilized, this point was thus being determined through the regression of the kernel weight as y = final kernel weight.

The efficiency of reserve mobilization (M_ε) was calculated as followed:

$$M_\varepsilon = \text{slope of regression II} \,/\, \text{slope of regression I} \tag{5}$$

$$\text{with } R_l = 1 - M_\varepsilon \tag{6}$$

being the resulting respiration losses (R_l) during mobilization of reserves.

3.4 Statistical analyses

3.4.1 Experiment I: Germination dynamics under variable thermal regimes

All test parameters are presented as the mean value ± standard error unless otherwise indicated. The indices to describe the germination dynamics were calculated using Excel 2013. All data were subjected to an analysis of variance (ANOVA) using the Mixed procedure of the Statistical Analyses System SAS® version 9.4 (random effects: block, growth chamber; fixed effects: cultivar, treatment) (see ANOVA tables in Appendix I). A residual analysis was carried out to verify normality and variance homogeneity. The experimental design layout was considered to be a split plot arrangement with main plots being organized in a row column design. Thus, the statistical model used was:

$$y_{ijkl} = \mu + \rho_i + c_j + (\rho * c)_{ij} + \nu_k + \tau_l + (\nu * \tau)_{kl} + \varepsilon_{ijkl} \tag{7}$$

where y_{ijkl} is the responsive variable, μ is the general mean, ρ_i is the i^{th} row effect, c_j is the j^{th} column effect, ν_k is the k^{th} cultivar effect, τ_l is the l^{th} treatment effect and ε_{ijkl} are random errors. Indices in percentage p are naturally rather binomially distributed than normally distributed. The following arcsine square root transformation helped therefore to achieve approximately normally distributed p-values:

$$y = \arcsin \sqrt{p} \tag{8}$$

Subsequently, the data was back transformed using the corresponding inverse function:

$$p = (sin(y))^2 \tag{9}$$

Means were compared based on the least significant test (LSD) using the statement LSMEANS/TDIFF option in PROC MIXED. Differences were considered significant when $P < 0.05$.

3.4.2 Experiment I: Dry matter partitioning and Experiment II: Reserve mobilization and dry matter partitioning in time course

To estimate the parameters for describing early growth processes like the onset of autotrophy, mobilization efficiency and the time until the plants compensated their initial kernel weight again a linear regression analysis was performed using SigmaPlot 12.5. The root, shoot and kernel dry weights obtained from the seedlings of Experiment I, as well as the parameters calculated on the basis of the regression lines, were then subjected to an analysis of variance (ANOVA) using the Mixed procedure of the Statistical Analyses System SAS® version 9.4 (see ANOVA tables in Appendix I and II). The statistical model used was:

$$y_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij} \tag{10}$$

where y_{ij} is the responsive variable, μ is the general mean, α and β the factors and ε the random error.

Means were then also compared using the least significant test (LSD) and differences were considered significant when $P < 0.05$. All Figures were done using SigmaPlot version 10.0 and 12.5.

4 RESULTS

4.1 Experiment I: Effects of temperatures on germination dynamics

Table 5
Parameter estimates (means and back-tranformed means) for effects of temperatures and cultivar on germination indices

Cultivar	Temp. (day/night in°C)	G [1] (%)	T_{50} [2] (days)	MGT [3] (days)	Z [4]
Chomrong	28/20	92.3 (±1.19)^{abcd}	3.2 (±0.30)^{c}	3.6 (±0.21)^{e}	0.54 (±0.06)^{cde}
	20/12	90.0 (±0.00)^{cd}	8.2 (±0.53)^{a}	8.3 (±0.30)^{cd}	0.44 (±0.04)^{def}
Faro 35	28/20	89.9 (±1.52)^{cd}	3.2 (±0.30)^{c}	3.8 (±0.24)^{e}	0.77 (±0.01)^{ab}
	20/12	82.3 (±4.91)^{d}	8.6 (±2.21)^{a}	9.3 (±0.29)^{a}	0.44 (±0.03)^{def}
IR 64	28/20	98.3 (±1.36)^{a}	3.6 (±0.28)^{c}	4.0 (±0.27)^{e}	0.71 (±0.02)^{abc}
	20/12	95.0 (±2.18)^{abc}	8.6 (±0.84)^{a}	9.1 (±0.23)^{ab}	0.85 (±0.03)^{a}
X 265	28/20	96.0 (±1.70)^{abc}	3.2 (±0.24)^{c}	3.6 (±0.23)^{e}	0.70 (±0.09)^{abcd}
	20/12	95.3 (±3.54)^{abc}	8.3 (±0.14)^{a}	8.6 (±0.05)^{bc}	0.51 (±0.08)^{de}
X jigna	28/20	98.3 (±0.82)^{a}	3.2 (±0.31)^{c}	3.8 (±0.31)^{e}	0.73 (±2.21)^{abc}
	20/12	97.3 (±2.37)^{ab}	7.5 (±0.30)^{ab}	8.3 (±0.19)^{cd}	0.36 (±0.07)^{ef}
Yun Keng	28/20	93.7 (±1.36)^{abcd}	3.2 (±0.03)^{c}	3.6 (±0.06)^{e}	0.59 (±0.06)^{bcd}
	20/12	88.3 (±2.36)^{bcd}	6.3 (±0.02)^{b}	7.7 (±0.04)^{d}	0.31 (±0.03)^{f}

[1] G = Germination in percentage; [2] T_{50} = Time taken to 50% germination of the seeds [Equation (1)]; [3] MGT = Mean germination time [Equation (2)]; [4] Z = Synchrony of germination [Equation (3)].
[a-f] Different letters indicate significant differences according to LSD at $P < 0.05$.

4.1.1 Germination percentage (G%)

Table 5 represents the results of the germination test (Experiment I). There was no great effect of temperature on the total number of germinated seeds. The germination percentage was reduced at 20/12°C day/night for all cultivars but did not differ significantly compared to optimal conditions. X jigna and IR 64 achieved with 98.3% the highest germination rate at 28/20°C day/night, while Faro 35 showed with 89.0% the lowest rate at this temperature regime. In addition, Faro 35 also showed the lowest germination percentage of 82.3% when exposed to low temperatures, which differed significantly from the cultivars IR 64, X 265 and X jigna. The least reduction was observed in X 265 with only 0.67% difference between the two temperature treatments. The highest reduction was displayed by Faro 35 with 7.6%. With 97.3%, X jigna had the highest germination rate at 20/12°C day/night. This was just 1% less than under optimal temperature conditions of 28/20 °C day/night.

4.1.2 Time taken to 50% germination of seeds (T_{50})

T_{50} is an index to determine the central tendency and represents the peak of germination [Equation (1)]. The results are also presented in table 5. It does not vary much between the cultivars within the same temperature treatment and no significant differences were observed. All cultivars displayed similar patterns and the time to reach T_{50} lengthened significantly at low temperature conditions. The cultivars reached T_{50} within 3.2-3.6 days. At low temperatures it took on average more than twice as much time (between 6.3 and 8.6 days) as under optimal conditions. T_{50} of Yun Keng (6.3 days) was significant lower compared to Chomrong (8.2 days), Faro 35 (8.6 days) and IR 64 (8.6 days).

4.1.3 Mean germination time (MGT)

The mean germination time (MGT) represents the average length of time to reach the maximum of germination and was calculated according to Equation (2). No significant differences could be detected between the cultivars when exposed to optimal temperature conditions. The cultivars displayed a MGT between 3.6 and 4.0 days.

However, MGT at low temperature conditions was significant extended (between 7.7 and 9.3 days) compared to optimal conditions. The MGT of the *japonica* cultivars Chomrong (8.3 days), X jigna (8.3 days) and Yun Keng (7.7 days) differed significantly from the MGT of the *indica* cultivars Faro 35 (9.3 days) and IR 64 (9.1 days).

4.1.4 Synchrony of germination (Z)

The synchrony of germination (Z) displays if one seed germinated together with another seed included in the same replication of one treatment [Equation (3)]. The closer Z to 1 the more seeds germinated at the same time and were correspondingly synchronous. At optimal conditions of 28/20°C day/night the cultivars showed higher germination synchrony than under low temperature conditions (between 0.54 and 0.77), with Faro 35 showing the highest Z value (0.77), which differed significantly from Chomrong (0.54). The cultivars Faro 35, X jigna and Yun Keng had significant lower Z values when exposed to low temperatures compared to optimal temperatures, with X jigna displaying the greatest differences with 0.37 between the two temperature treatments. In contrast, Chomrong differed only by 0.10.

4.1.5 Germination percentage in time course

Figure 14 gives a closer look at the time course of the cumulative germination percent of the different cultivars at the two relevant temperature regimes (a and b). The time to reach the maximum of germination percentage was significantly extended at temperatures of 20/12°C day/night compared to temperatures of 28/20°C day/night. All cultivars reached their maximum of germination already within approximately five days at optimal conditions, while it also took around five days to the onset of germination at 20/12°C day/night. At temperatures of 28/20°C day/night, the differences between the cultivars were marginal and did not differ significantly from each other. At low temperatures on the other hand, differences between the cultivars were more obvious. The onset of germination was the earliest for Yun Keng in both temperature treatments. However, the germination of the seeds was relatively distributed, as evidenced by the slightest slope of its curve. In addition, the percentage of germination still increased after 14 days at 20/12°C day night, while the other cultivars reached their maximum within twelve days. The *indica* cultivars IR 64 and Faro 35 showed a significant delay in initiating the germination process at low temperatures. However, all seeds germinated relatively synchronous, as evidenced by the enormous slope of its curve. Hence, this figure demonstrates, that the onset of germination is delayed when seeds were exposed to low temperatures but it did not influence germination percentage. As soon as the first seeds started to germinate, the curves followed almost the same pattern in both temperature treatments.

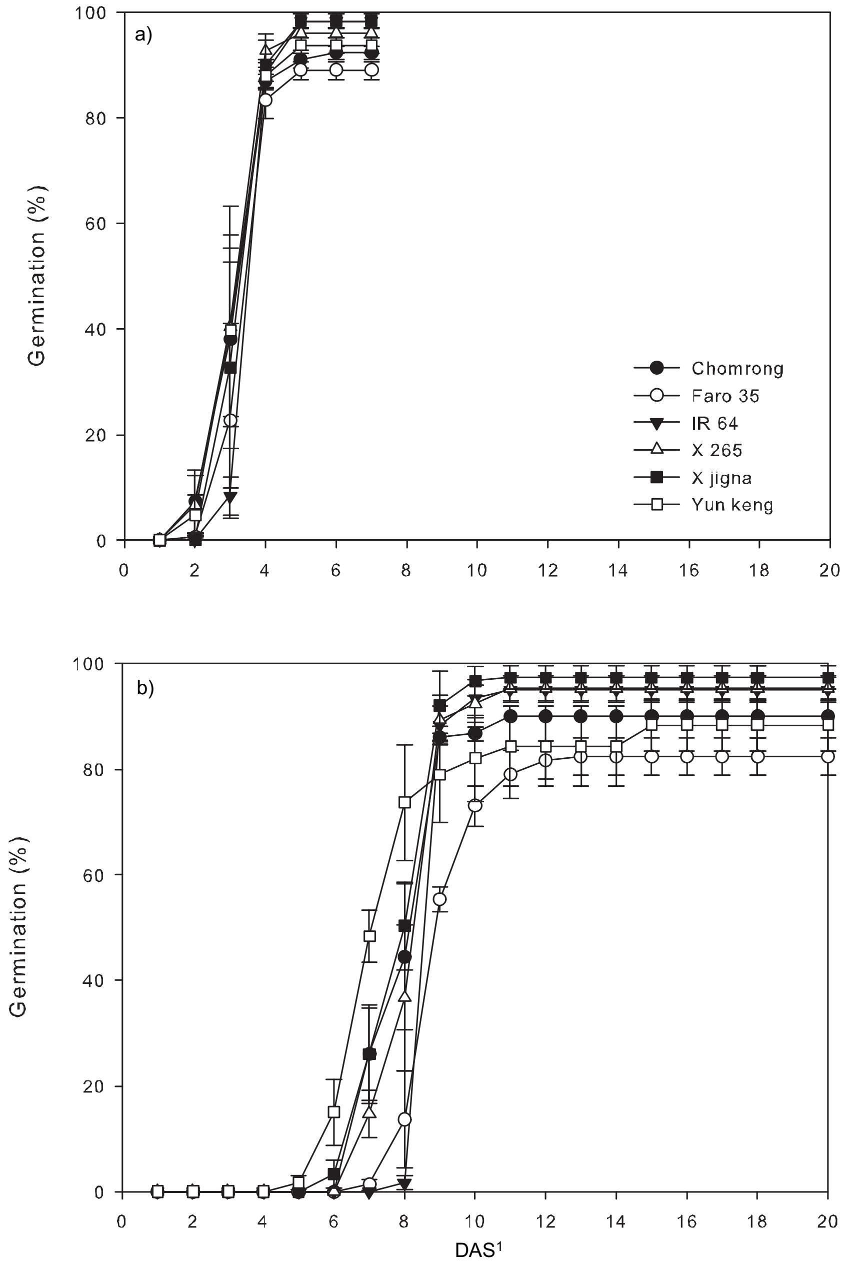


Figure 14 Time courses of cumulative germination percentage of the six different cultivars; a) seeds germinating at 28/20 °C day/night and b) seeds germination at 20/12 °C day/night. [1]DAS = days after sowing; error bars indicate standard error of mean.

4.2 Experiment I: Effect of temperature on dry matter partitioning

4.2.1 Dry matter distribution

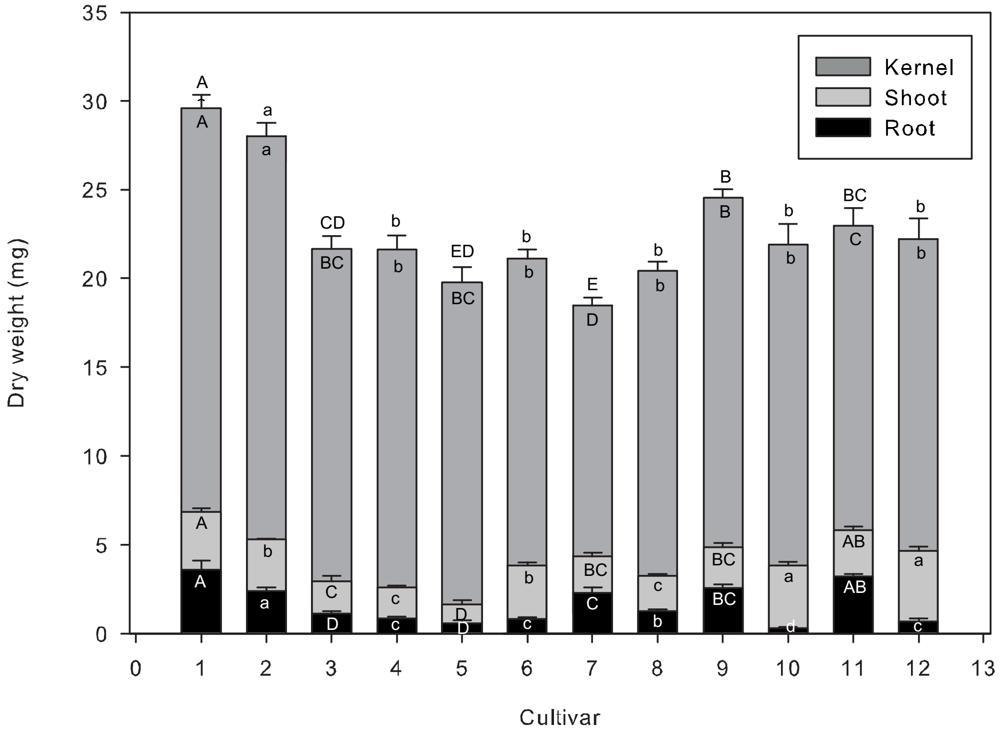


Figure 15 Dry weight distribution in mg of six different cultivars at two temperature regimes after seven days at 28/20°C day/night and 20 days at 20/12°C day/night. 1= Chomrong at 28/20°C day/night; 2= Chomrong at 20/12°C day/night; 3= Faro 35 at 28/20°C day/night; 4= Faro 35 at 20/12°C day/night; 5= IR 64 at 28/20°C day/night; 6= IR 64 at 20/12°C day/night; 7= X 265 at 28/20°C day/night; 8= X 265 at 20/12°C day/night; 9= X jigna at 28/20°C day/night; 10= X jigna at 20/12°C day/night; 11= Yun Keng at 28/20°C day/night; 12= Yun Keng at 20/12°C day/night. Error bars indicate standard error of mean.

$^{A-E}$ Different capital letters indicate significant differences between cultivars growing at 28/20°C day/night at $P < 0.05$. $^{a-d}$ Different small letters indicate significant differences between cultivars growing at 20/12°C day/night at $P < 0.05$. Letters apply from top to bottom for: total dry weight, kernel dry weight, shoot dry weight, root dry weight.

The effect of cultivar on dry weights (DWs) of roots, shoots and kernels at two temperature regimes is shown in Figure 15. Considering the total DWs of the cultivars at optimal temperature conditions of 28/20°C day/night (bar 1, 3, 5, 7, 9, 11) differences between the *japonica* cultivars Chomrong, X jigna and Yun Keng and the *indica* cultivars Faro 35 and IR 64 were observed. Chomrong and X jigna displayed significant higher total DWs than the *indica* cultivars. At low temperatures (bar 2, 4, 6, 8, 10, 12) only Chomrong achieved a significant higher total DW than the other cultivars tested. Comparing the two temperature treatments it becomes apparent, that the total DWs are very similar within one cultivar, which indicates that it took approximately 13 days longer for plants growing at low temperature conditions to achieve the same seedling DW than for plants growing at optimal temperatures.

The remaining kernel DWs at optimal temperature conditions as well as at low temperature conditions just reflecting their initial kernel weights (Tab. 6). Chomrong with the highest initial kernel DW of 32.0 mg displayed also the highest remaining kernel DW in both treatments. The remaining kernel DWs of one cultivar did not differ much between the two temperature regimes, only X 265 showed an increased kernel weight loss at optimal temperature conditions. Root DW were higher at optimal temperature conditions of 28/20°C day/night only IR 64 showed a slightly higher root DW when exposed to low temperatures. Root DWs were significant higher for the *japonica* cultivars Chomrong, X jigna and Yun Keng compared to the *indica* cultivars Faro 35 and IR 64 at optimal conditions. Chomrong, X 265, X jigna and Yun Keng showed a greatly decrease in root DW at low temperatures as compared to the root DWs of the seedlings growing at optimal conditions. However, Chomrong displayed still the highest root DW when exposed to low temperature conditions, which differed significantly from the other cultivars growing at same temperatures. Minimal root dry weights were recorded for X jigna, Yun Keng and Faro 35 when exposed to 20/12°C day/night, as well as for IR 64 at optimal conditions of 28/20°C day/night.

However, X jigna and Yun Keng appeared to compensate the minor root development with increased shoot growth when exposed to low temperatures, which is indicated by the highest shoot DWs and a very low root/shoot DW ratio (Fig. 16) for both cultivars.

In addition to the higher root DW at 20/12°C day/night, the cultivar IR 64 also showed a higher shoot DW compared to low temperature conditions. Chomrong, Faro 35 and X 265 revealed hardly any differences in shoot DW when comparing the two temperature treatments. The highest shoot DW at optimal temperature conditions was recorded for Chomrong, which differed significantly from the other cultivars tested. At low temperature conditions the highest shoot DW was recorded for Yun Keng.

4.2.2 Root/Shoot dry weight ratio

Considering the root/shoot DW ratios, represented in Figure 16, it becomes apparent, that all tested cultivars displayed higher root/shoot ratios at optimal temperature conditions as compared to seedlings exposed to 20/12°C day/night.

At optimal temperature conditions, Chomrong, X 265 and Yun Keng had significant higher root/shoot ratios as compared to Faro 35 and IR 64. The lowest root/shoot ratios at low temperatures were recorded for X jigna and Yun Keng. Chomrong and Faro 35 displayed only minor differences between the two temperature treatments as compared to the other cultivars tested.

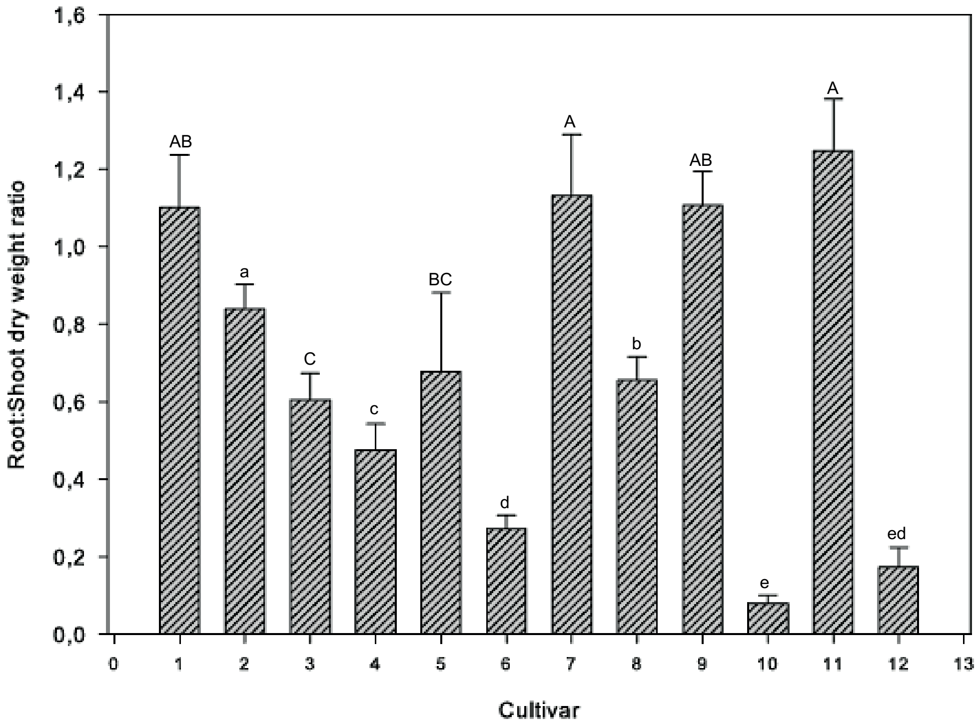


Figure 16 Root: Shoot dry weight ratio of six different cultivars at two temperature regimes after seven days at 28/20°C day/night and 20 days at 20/12°C day/night. 1= Chomrong at 28/20°C day/night; 2= Chomrong at 20/12°C day/night; 3= Faro 35 at 28/20°C day/night; 4= Faro 35 at 20/12°C day/night; 5= IR 64 at 28/20°C day/night; 6= IR 64 at 20/12°C day/night; 7= X 265 at 28/20°C day/night; 8= X 265 at 20/12°C day/night; 9= X jigna at 28/20°C day/night; 10= X jigna at 20/12°C day/night; 11= Yun Keng at 28/20°C day/night; 12= Yun Keng at 20/12°C day/night. Error bars indicate standard error of mean.

$^{A\text{-}C}$ Different capital letters indicate significant differences between cultivars growing at 28/20°C day/night at $P < 0.05$. $^{a\text{-}e}$ different small letters indicate significant differences between cultivars growing at 20/12°C day/night at $P < 0.05$.

4.2.3 Weight of mobilized seed reserves, seed reserve utilization efficiency and seed reserve depletion percentage

Table 6 represents the results of the calculation of the weight of mobilized seed reserves (WMSR), the seed reserve mobilization efficiency (SRUE) as well as the seed reserve depletion percentage (SRDP) after 7 days for seedlings growing at 28/20°C day/night and after 20 days for seedlings growing at 20/12°C day/night. In addition, the initial kernel DW, the final kernel DW and the seedling DW (root and shoot) (SLDW) are also listed in table 6.

The initial kernel DW before soaking was between 23.4 and 32.0 mg and differed significantly for all cultivars. Higher kernel DW were recorded for the *japonica* cultivars Chomong, X jigna and Yun Keng as compared to other cultivars tested in this study.

The weight of mobilized seed reserves (WMSR) differed greatly between the cultivars at optimal temperatures. The highest WMSR of 10.9 mg was recorded for Yun Keng and differed significantly from Faro 35 (5.5 mg), IR 64 (7.7 mg) and X jigna (7.7 mg). At low temperature conditions, each cultivar displayed similar WMSRs as compared to optimal temperature conditions and Yun Keng showed as well the highest WMSR (10.6 mg) which differed significantly from Faro 35 (5.2 mg) and X 265 (6.3 mg). The related WMSR in both treatments indicated, that the seedlings growing at low temperatures mobilized a similar amount of seed reserves with a lag of about 13 days.

SLDW was highest for Chomrong (6.8 mg) and Yun Keng (5.8 mg) both differing significantly from Faro 35 (3.0 mg), IR 64 (1.6 mg) and X 265 (4.4 mg) at optimal temperatures of 28/20°C day/night. The seedlings obtained slightly less DWs at low temperatures after 20 days of growth compared to seven days at optimal temperature conditions. Only IR 64 displayed a higher SLDW at low temperatures as compared to optimal temperatures.

The *japonica* cultivars Chomrong, X jigna and Yun Keng obtained on average higher SLDWs as compared to the *indica* cultivars Faro 35 and IR 64.

SRUE, which predicates the conversion efficiency of seed reserves into plant tissue was highest for Chomrong (0.74) at optimal temperature conditions and differed significantly from the *indica* cultivars Faro 35 (0.54) and IR 64 (0.21), as well as X 265 (0.45) and Yun Keng (0.53). At low temperature conditions, SRUE was decreased for the majority of the cultivars, only IR 64 and X 265 showed slightly higher SRUE compared to the SRUE at optimal temperature conditions. The highest SRUE at low temperatures was again recorded for Chomrong (0.57), which differed significantly from Faro 35 (0.50), IR 64 (0.45), X jigna (0.41) and Yun Keng (0.44).

SRDP was significant higher for X 265 (40%) and Yun Keng (39%) as compared to the other cultivars at optimal temperature conditions. Faro 35 depleted its seed reserves to only 21% at low temperatures which differed significantly from Yun Keng (37%), X jigna (34%) and IR 64 (33%).

Table 6
Parameter estimates (means and back-transformed means ± standard errors) for the effects of temperature and cultivar on weight of mobilized seed reserves, seed reserve utilization efficiency and seed reserve depletion percentage after 7 days for plants growing at 28/20°C day/night and after 20 days for plants growing at 20/12°C day/night

Cultivar	Treatment (°C day/night)	Initial kernel weight (mg)	Final kernel weight (mg)	WMSR[1] (mg)	SLDW[2] (mg)	SRUE[3] (mg)	SRDP[4] (%)
Chomrong	28/20	32.0 (±0.19)^{A}	22.8 (±0.79)^{A}	9.2 (±0.79)^{AB}	6.8 (±0.68)^{A}	0.74 (±0.07)^{A}	0.28 (±0.01)^{B}
	20/12	32.0 (±0.19)^{a}	22.7 (±0.77)^{a}	9.3 (±0.77)^{a}	5.3 (±0.22)^{a}	0.57 (±0.02)^{a}	0.29 (±0.02)^{ab}
Faro 35	28/20	24.2 (±0.13)^{E}	18.7 (±0.71)^{BC}	5.5 (±0.71)^{C}	3.0 (±0.40)^{E}	0.54 (±0.07)^{BC}	0.23 (±0.03)^{B}
	20/12	24.2 (±0.13)^{e}	19.0 (±0.79)^{b}	5.2 (±0.79)^{c}	2.6 (±0.18)^{d}	0.50 (±0.03)^{bc}	0.21 (±0.03)^{b}
IR 64	28/20	25.8 (±0.12)^{D}	18.1 (±0.89)^{BC}	7.7 (±0.89)^{BC}	1.6 (±0.26)^{D}	0.21 (±0.03)^{D}	0.30 (±0.03)^{B}
	20/12	25.8 (±0.12)^{d}	17.3 (±0.49)^{b}	8.5 (±0.49)^{ab}	3.8 (±0.19)^{c}	0.45 (±0.03)^{bcd}	0.33 (±0.02)^{a}
X 265	28/20	23.4 (±0.13)^{D}	14.1 (±0.44)^{BC}	9.3 (±0.44)^{AB}	4.4 (±0.41)^{C}	0.45 (±0.04)^{C}	0.40 (±0.02)^{A}
	20/12	23.4 (±0.13)^{f}	17.2 (±0.49)^{b}	6.3 (±0.49)^{bc}	3.3 (±0.10)^{c}	0.52 (±0.02)^{ab}	0.27 (±0.02)^{ab}
X jigna	28/20	27.4 (±0.16)^{C}	19.7 (±0.49)^{B}	7.7 (±0.49)^{BC}	4.9 (±0.43)^{BC}	0.63 (±0.06)^{AB}	0.28 (±0.02)^{B}
	20/12	27.4 (±0.16)^{c}	18.1 (±1.16)^{b}	9.3 (±1.16)^{a}	3.8 (±0.22)^{c}	0.41 (±0.02)^{d}	0.34 (±0.04)^{a}
Yun Keng	28/20	28.1 (±0.15)^{B}	17.2 (±0.97)^{C}	10.9 (±0.97)^{A}	5.8 (±0.22)^{AB}	0.53 (±0.02)^{BC}	0.39 (±0.03)^{A}
	20/12	28.1 (±0.15)^{b}	17.6 (±1.16)^{b}	10.6 (±1.16)^{a}	4.7 (±0.23)^{b}	0.44 (±0.02)^{cd}	0.37 (±0.04)^{a}

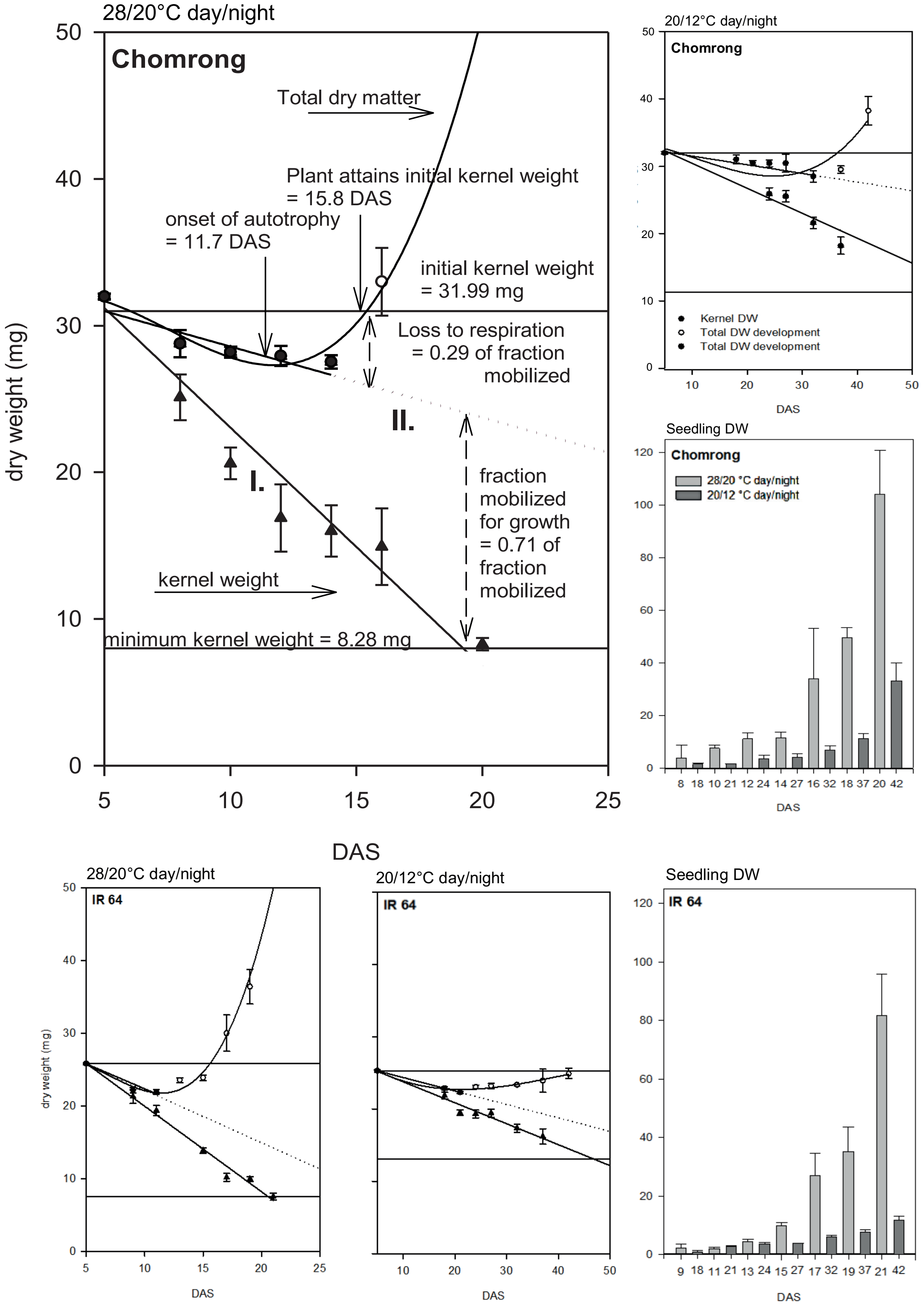
[1] WMSR = weight of mobilized seed reserves [2] SLDW = Seedling dry weight; [3] SRUE = seed reserve mobilization efficiency; [4] SRDP = seed reserve depletion percentage; mean values were calculated from ten repetitions.

$^{A-E}$ Different capital letters indicate significant differences between cultivars growing at 28/20°C day/night at $P < 0.05$.

$^{a-f}$ Different small letters indicate significant differences between cultivars growing at 20/12°C day/night at $P < 0.05$.

4.3 Experiment II: Effect of temperature on seed reserve mobilization and dry matter partitioning

4.3.1 Mobilization of seed reserves and seedling growth over time



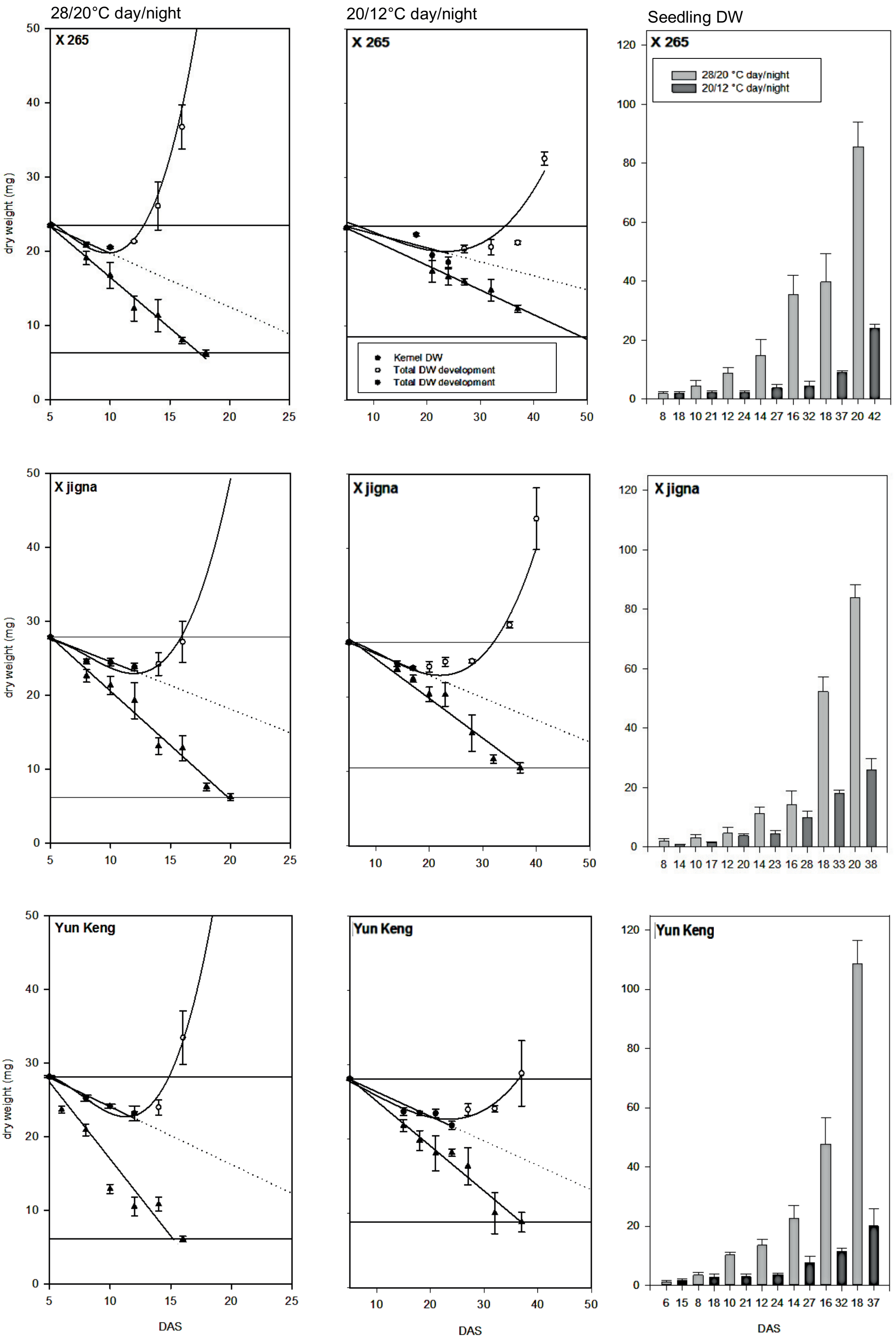


Figure 17 Linear regression analyses performed to estimate the mobilization efficiency, the onset of autotrophy and the time until the plant attains its initial kernel weight. Triangles: kernel weight decrease = data used for regression I; Circles: Total dry weight development. Curve were fitted with third order regression. Gray shaded circles = data used for regression II. Error bars = standard error of mean. Upper reference line = initial kernel weight, lower reference line = minimum kernel weight. Corresponding linear regression equations are presented in Table 7. Seedling weight is the sum of DW of root, shoot and leaf. DAS = days after sowing.

Figures 17 shows the linear regression analyses performed to determine the mobilization efficiency, the days to onset of autotrophy as well as the time until the seedlings compensated their initial kernel weight again. The corresponding equations are represented in table 7. In addition, the seedling dry weight (root, shoot and leaf) for each day of sampling is diagramed.

When exposed to optimal temperatures of 28/20°C day/night, the total DW (roots, shoots, leaves and remaining kernels) decreased between 10.9% for X 265 and 17.7% for Yun Keng during the initial 9.9-12.8 days after sowing (DAS) (Fig. 18) which characterizes the heterotrophic growth phase (Tab. 8). This initial DW loss was compensated between 12.8 (X 265) and 16.1 (IR 64) DAS with X 265 and Yun Keng attaining the intial kernel DW significantly faster than the other cultivars. The kernel DW decreased linear until the end of the experiment and levelled off at 22.6-29.1% of the initial kernel DW. Correspondingly, between 71 and 78% of the kernel DW was mobilized during the germination process, with Yun Keng showing a significant higher kernel DW loss of 78% compared to X 265 with 73% and IR 64 with 71%. In general in can be stated that the *japonica* cultivars displayed a higher kernel DW losses than the *indica* cultivars.

Considering the relative rates of mobilization, only Yun Keng showed a quicker kernel DW decrease than the other cultivars and its DW decreased to half of its initial value within 10 days, while it took between 14-15 days for the other cultivars tested in this experiment. However, the onset of photo-autotrophy did not coincide with the end of seed reserve mobilization, but reserves lasted even beyond the time when the plant attained its initial kernel DW again. The mobilization efficiency [Equation (5)] revealed only minor differences between the cultivars exposed to optimal temperature conditions. The cultivars Chomrong, X 265 and X jigna showed similar mobilization efficiencies between 0.69-0.71 which differed significantly from IR 64 with an efficiency of only 0.55. The higher the seed reserve mobilization efficiency, the more seed reserves are converted directly into growth and respiration losses are decreased. This was also evident in the seedlings DW development (right figure) which was higher for cultivars with higher mobilization efficiencies. Seedling DW increased by between 1.9 mg (IR 64) and 7.5 mg (Yun Keng) from the beginning of the experiment (initial kernel weight) to the end of heterotrophic phase, or by a kernel weight loss of 23.2% (IR 64) to 52.0% (Yun Keng).

Kernel DW loss [Equation (4)], mobilization efficiency, time to onset of autotrophy as well as time until the plant attains its initial kernel DW differed greatly for the two temperature treatments. When exposed to low temperatures of 20/12°C day/night, the total DW (root, shoot, leaves and remaining kernel) decreased between 11% for Chomrong and 22.3% for Yun Keng during the initial 20.2-26.8 days after sowing (DAS) (Fig.18), with Yun Keng and X 265 losing more weight during heterotrophic phase at low temperature treatment than the other cultivars tested. The fraction of material mobilized from the seed was less in all cultivar when exposed to low temperatures, with IR 64 mobilizing only 49% which differed significantly from the other cultivars with a kernel DW loss between 62% for X jigna and 69% for Yun Keng. Considering the relatives rates of mobilization, Yun Keng also showed a quicker kernel DW decrease at this temperature regime than the other cultivars and its DW decreased to half of its initial value within around 28 days, while it took even more than 40 days for Chomrong. The time until the plants became photo-autotroph was on average more than twice as much as under optimal temperatures. The *indica* genotypes IR 64 reached after 20.2 days the photo-autotrohpic phase, while it took 26.8 days for X 265 to become photo-autotrophic. The mobilization efficiency was reduced during low temperature treatment for all cultivars, which resulted correspondingly in higher respiration losses [Equation (6)]. However, the mobilization efficiency of Chomrong was only slightly reduced compared to X 265. IR 64 and X 265 displayed the lowest mobilization efficiency of 0.43, which differed significantly from Chomrong, and X jigna. The different mobilization efficiencies were also reflected in the seedlings DW again and cultivars with higher mobilization efficiencies achieved higher seedlings DW during the entire experiment. It took on average more than twice as much time to reach the initial kernel DW again for the plants exposed to low temperatures and no significant differences between the cultivars could be recorded. Chomrong was the fastest with 34.7 days while it took 36.7 days for X 265 to attain its initial weight again. However, this time coincides almost with the end of seed reserve mobilization for the cultivars IR 64, X jigna and Yun Keng, while the seed reserves lasted even beyond this time for the cultivars Chomrong and X 265.

Table 7
Linear Regression of kernel weight decrease (Regression I) and the initial total dry weight losses during the heterotrophic phase (Regression II) used to determine the mobilization efficiency of six different cultivars at 28/20°C day/night and 20/12°C day/night

Cultivar	Temp. (day/night in °C)	Regression I[1] (mg/day)	r^2	Regression II[1] (mg/day)	r^2
Chomrong	28/20	$39.4-1.63x$	0.97	$33.4-0.48x$	0.83
	20/12	$34.8-0.40x$	0.93	$32.8-0.14x$	0.94
Faro 35	28/20	-	-	-	-
	20/12	-	-	-	-
IR 64	28/20	$31.7-1.24x$	0.99	$29.3-0.59x$	0.99
	20/12	$26.7-0.31x$	0.98	$26.2-0.18x$	1.00
X 265	28/20	$30.1-1.51x$	0.99	$26.2-0.47x$	0.92
	20/12	$25.2-0.35x$	0.98	$24.3-0.20x$	0.77
X jigna	28/20	$35.2-1.72x$	0.99	$30.9-0.52x$	0.92
	20/12	$30.6-0.60x$	0.97	$28.9-0.24x$	0.99
Yun Keng	28/20	$37.9-2.14x$	0.95	$32.3-0.80x$	0.98
	20/12	$31.2-0.65x$	0.97	$29.7-0.30x$	0.97

[1] Linear regression analyses were performed using SigmaPlot 12.5.
[2] r^2= Coefficient of determination.

Table 8
Parameter estimates (means ± standard error) for the effects of temperature and cultivar on kernel dry weight loss, mobilization efficiency, time taken to onset of autotrophy and time taken until plant attains initial kernel dry weight.

Cultivar	Treatment (°C day/night)	Initial kernel weight (mg)	Kernel DW loss[1] (DW/DW)	Mobilization efficiency[2] (DW/DW)	Respiration loss[3] (DW/DW)	Onset of autotrophy (DAS)	Plant attains initial kernel DW (DAS)
Chomrong	28/20	32.0 (±0.19)^{A}	0.74 (±0.01)^{ABC}	0.71 (±0.04)^{A}	0.29 (±0.04)^{B}	11.7 (±0.58)^{AB}	15.1 (±0.28)^{A}
	20/12	32.0 (±0.19)^{a}	0.65 (±0.04)^{a}	0.65 (±0.04)^{a}	0.35 (±0.04)^{b}	26.7 (±1.12)^{a}	34.7 (±1.44)^{a}
Faro 35	28/20	24.2 (±0.13)^{E}	-	-	-	-	-
	20/12	24.2 (±0.13)^{e}	-	-	-	-	-
IR 64	28/20	25.8 (±0.12)^{D}	0.71 (±0.02)^{C}	0.55 (±0.04)^{B}	0.45 (±0.04)^{A}	12.8 (±0.42)^{A}	16.1 (±0.37)^{A}
	20/12	25.8 (±0.12)^{d}	0.49 (±0.01)^{b}	0.43 (±0.03)^{c}	0.57 (±0.03)^{a}	20.2 (±2.81)^{b}	36.3 (±2.49)^{a}
X 265	28/20	23.4 (±0.13)^{F}	0.73 (±0.02)^{BC}	0.69 (±0.11)^{A}	0.31 (±0.11)^{B}	9.90 (±0.66)^{C}	12.8 (±0.65)^{B}
	20/12	23.4 (±0.13)^{f}	0.63 (±0.01)^{a}	0.43 (±0.07)^{c}	0.57 (±0.07)^{a}	26.8 (±1.50)^{a}	36.9 (±1.38)^{a}
X jigna	28/20	27.4 (±0.16)^{C}	0.77 (±0.01)^{AB}	0.70 (±0.03)^{A}	0.30 (±0.03)^{B}	12.0 (±0.18)^{A}	15.2 (±0.26)^{A}
	20/12	27.4 (±0.16)^{c}	0.62 (±0.03)^{a}	0.60 (±0.03)^{b}	0.40 (±0.03)^{b}	24.9 (±1.04)^{ab}	35.2 (±0.80)^{a}
Yun Keng	28/20	28.1 (±0.15)^{B}	0.78 (±0.01)^{A}	0.63 (±0.02)^{AB}	0.37 (±0.02)^{ab}	10.5 (±0.29)^{BC}	13.4 (±0.32)^{B}
	20/12	28.1 (±0.15)^{b}	0.69 (±0.04)^{a}	0.53 (±0.02)^{bc}	0.47 (±0.02)^{ab}	24.6 (±1.53)^{ab}	35.1 (±1.65)^{a}

[1] Kernel dry weight loss was calculated according to Equation (4). [2] Mobilization efficiency was calculated according to Equation (5). [3] Respiration loss was calculated according to Equation (6). DW = dry weight; DAS = days after sowing.
$^{A-F}$ Different capital letters indicate significant differences between cultivars growing at 28/20°C day/night at $P < 0.05$.
$^{a-c}$ Different small letters indicate significant differences between cultivars growing at 20/12°C day/night at $P < 0.05$.

4.3.2 Time course of dry weights of root, shoot, leaf, kernel and total seedling

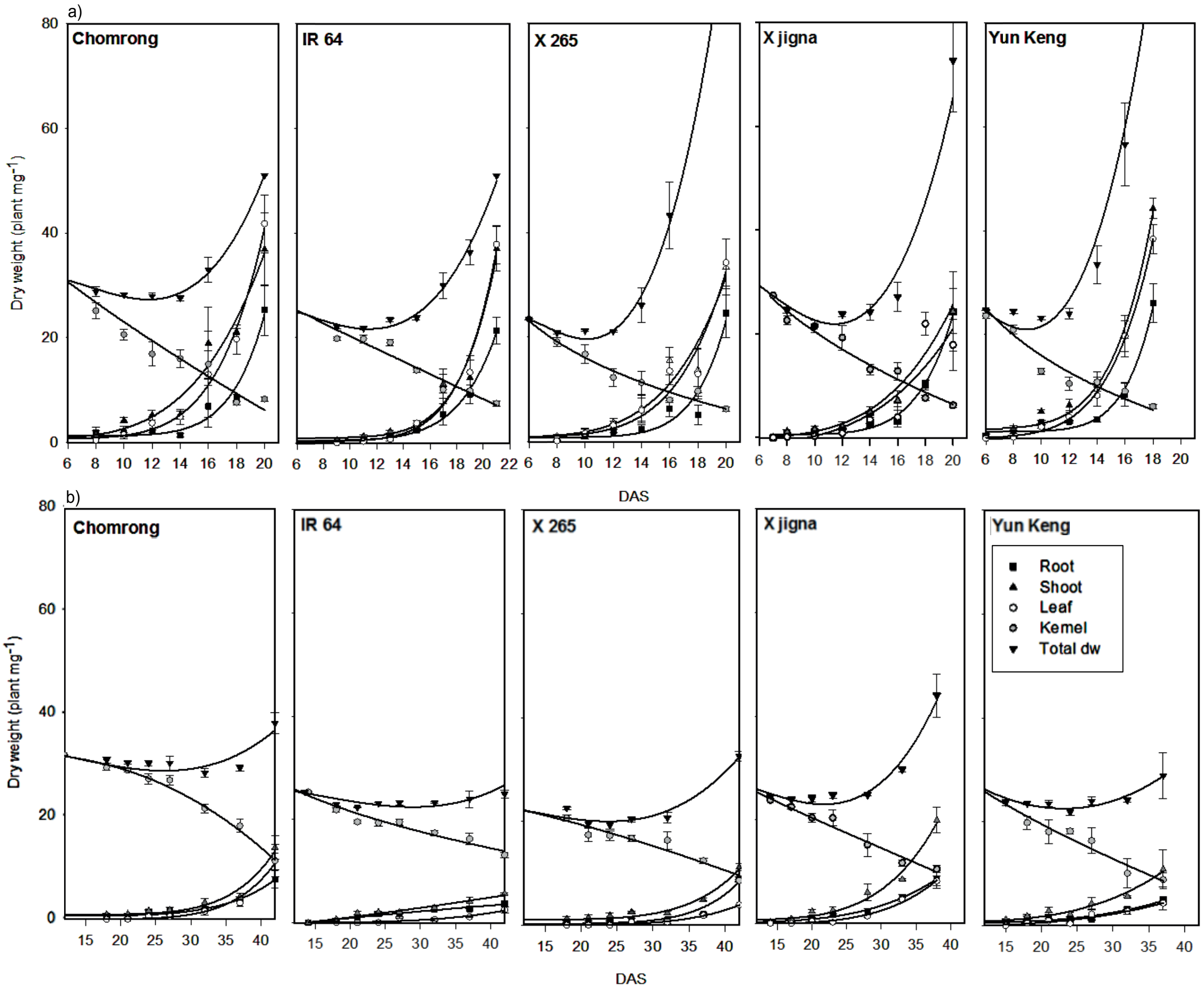


Figure 18 Time courses of dry weights of root, leaf, shoot, kernel and total seedling for five rice cultivars at 28/20°C day/night (a) and 20/12°C day/night (b). DAS = days after sowing. Lines were fitted with third-power regressions.

The dry weight growth of the individual plant organs root, shoot, leaves as well as kernel DW and the total seedling DW in the course of time of the five different cultivars at two temperature regimes is shown in Figure 18. The growth of the individual organs of the seedling was mostly greatest for leaves, closely followed by shoots and smallest for roots for the plants growing at optimal conditions of 28/20°C day/night (top figures). Only X jigna behaved different with shoot growth being as great as root growth followed by the leaves during the entire experiment period. The patterns of growth were generally exponential, but no further root growth could be observed for the period when the seedlings became photo-autotrophic (9.9-12.8 DAS). The cultivars Yun Keng and Chomrong displayed higher dry weights of leaves, shoots and roots, followed by IR 64. Smallest DWs were observed for X 265 and X jigna.

During the low temperature treatment (bottom figures) the increase in growth for the individual plant organs was much slower compared to seedlings growing at optimal conditions and unlike those plants, cold treated ones showed the greatest DW growth for shoots, followed by roots and smallest for leaves. The highest DWs were observed for X jigna and Chomrong, X 265 and Yun Keng were intermediate, while IR 64 displayed the smallest DWs for the individual plant organs.

4.3.3 Dry matter partitioning among organs

In order to understand if the observed patterns of root, shoot and leaf growth were due to changes in overall growth or due to an adaption strategy to tolerant low temperatures, the ratio of each individual organ to the total seedling dry weight was calculated for each sampling date for the different cultivars and is shown in Figure 19.

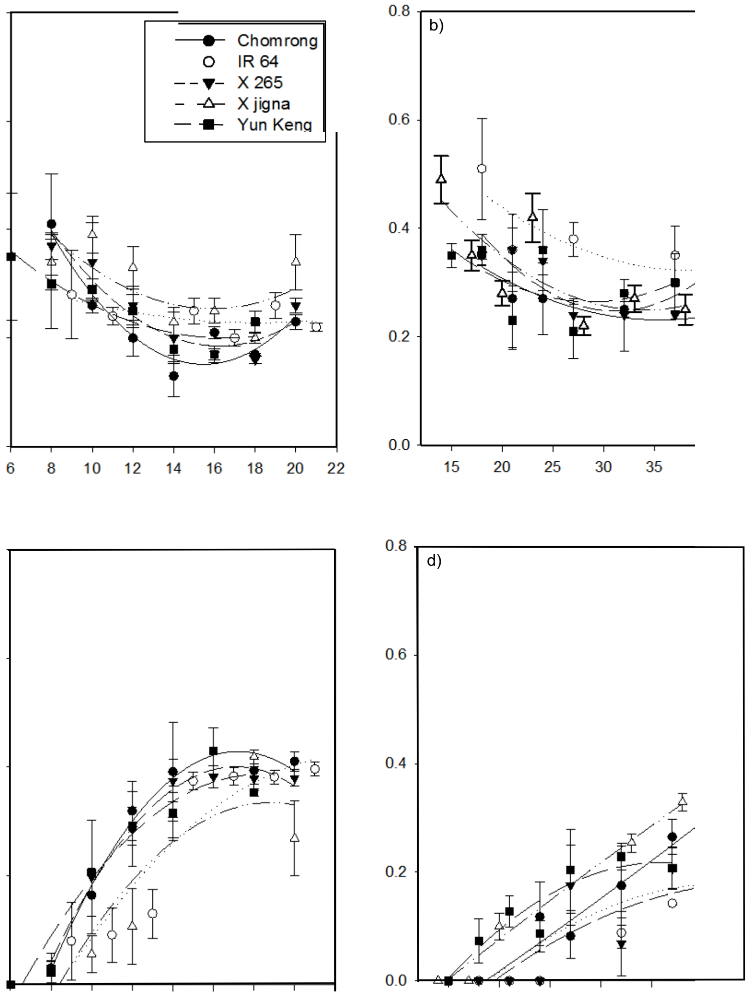


Figure 19 Time courses of dry matter distribution in rice seedlings. a) root/plant dry weight ratio for five ric cultivars growing at 28/20°C day/night; b) root/plant dry weight ratio for five rice cultivars growing at 20/12°(day/night; c) leaf/plant weight ratio for five rice cultivars growing at 28/20°C day/night and d) leaf/plant ratio fc

The cultivars showed similar patterns for DW ratios between roots and the total seedlings DW, as well as between leaf blades and the total seedling DW when exposed to optimal conditions of 28/20°C day/night (left figures). The DW fraction, which was found in the root system was between 0.35-0.45 for Yun Keng and Chomrong at the first date of sampling. Afterwards, it decreased constantly to 0.18-0.28 for Chomrong and IR 64 until 15 DAS and increased again between 16-18 DAS. The decrease of the root system coincided with the increased development of leaves (Figure c), as well as with the outage the seed reserves. The leaf fraction was still 0 at the beginning of sampling but increased constantly to 0.30-0.40 for IR 64 and Chomrong until around 16 DAS. Slighlty higher DW fractions in leaves were recorded for Chomrong and Yun Keng.

During low temperature treatment, the cultivars displayed an increased scatter but still showed similar patterns for DW ratios between roots and the total seedling DW and between leaf blades and the total seedling DW (right figures). The DW fraction for roots were similar as when exposed to optimal conditions and showed values between 0.38-0.5 at the beginning of sampling but dropped unlike the plants exposed to optimal temperature treatment to just about 0.3-0.38 for Chomrong and IR 64 until 30 DAS and increased slowly at around 35 DAS again. Unlike rice plants exposed to optimal conditions, this low point in root growth does not coincides with the end of seed reserve mobilization, but with the onset of autotrophy. The decrease of the root system coincided also with an increased development of the photosynthesis apparatus (Figure d). The greatest decrease in root growth was recorded for Chomrong and X jigna, which resulted in the largest increase in leaf development.

5 DISCUSSION

5.1 Effect of low temperature and cultivar on germination dynamics and dry matter partitioning

Low temperatures negatively affect both, productivity and yield of rice grown in colder regions. Especially direct-sown rice plants need a longer growth duration as compared to transplanted seedlings. To master this challenge, early sowing should be made and germination and seedling growth should begin as soon as possible in order to establish productive rice plants (Sasaki, 1983). Thus, it is important to identify rice cultivars which are less sensitive to low temperatures and have a higher seed and seedling vigor. Germination percentage (G%), the time until 50% of the seeds are germinated (T_{50}) as well as the mean germination time (MGT) have been widely used to evaluate seed vigor of plants (Ranal and Santana 2006). In addition, they are very useful parameters for evaluating cold tolerance of rice cultivars in this early stage of growth (Ye et al., 2009). However, the results from this study indicate, that the germination percentage on its own represents no significant evaluation index in screening cultivars for cold tolerance at an average temperature of 16°C. In regard to germination percentage, no significant differences between the two temperature treatments were observed and even the differences between the cultivars were variable and no clear trend between *japonica* cultivars (Chomrong, Yun Keng and X jigna), which are known to tolerate cold better and *indica* cultivars (Faro 35, IR 64) were recorded. On the other hand, T_{50} and MGT showed highly significant differences between the two temperature treatments due to a delayed onset of germination when seeds were exposed to low temperatures. Once the first seeds started to germinate they followed almost the same pattern as under optimal temperature conditions. This result is also confirmed by Ye et al. (2009). He also revealed that no correlation exists between germination percentage and germination speed and therefore advised to evaluate cold tolerance by collecting several parameters regarding the entire germination process.

The present study also proved differences between *japonica* and *indica* cultivars in regard to T_{50} and MGT. It took longer for *indica* cultivars to reach T_{50} and MGT, with MGT values being significantly higher than those of *japonica* cultivars. Although it is believed that *japonica* and *indica* sub-species have the same origin, they were still domesticated differently (Khush, 1997). *Japonica* cultivars are mainly cultivated in temperate regions at high latitudes or altitudes, whereas the *indica* rice ecotypes are predominantly cultivated in subtropical regions with either low latitude or altitude (Lu et al., 2009). Therefore, it is assumed that the *japonica* ssp. is better adapted to low temperatures than *indica* ssp. and possess a stronger seed vigor in response to germination speed.

This result is also supported by the outcome of Zakarias et al. (2001) experiment, in which a reduction in the speed of germination in rice seeds at 17°C was observed. They furthermore verified, that at this temperature regime the surface of starch granules differed from that at optimal temperature conditions, which might be due to a low activity or even a lack of degradation enzymes. Sanwo and DeMason (1992), who examined also the germination dynamics in sweet corn at low temperatures, concluded that this is due to a delayed starch degradation based on an insufficient production of α-amylase in the scutellum of the seeds at low temperatures. The depletion of sugars in the embryo as well as gibberelic acid (GA_3) in the seeds stimulate the syntheses and activation of hydrolytic enzymes such as α-amylase, while on the other hand the presence of sugars in the embryo as well as the stress hormone abscisic acid (ABA), suppresses the syntheses of α-amylase (Mayer and Poljakoff-Mayber, 1989; Perata et al., 1997). In the present study it can be therefore assumed, that the delayed onset of germination during low temperature treatment is due to a delayed degradation of starch based on an insufficient production of α-amylase, which is most likely caused by the production of the stress hormone ABA.

It has been also reported that the seed size and seed weight are associated with seed vigor in rice (Chen et al., 2015; Lu et al., 2007). Huang et al. (2017) also supports the hypotheses, that rice cultivars with a higher seed amylose content and amylase activity may have superior seed vigor. In the present study the initial seed DWs of the various cultivars differed significantly, with higher initial seed DWs for *japonica* than for *indica* genotypes. Hence, it could be concluded, that *japonica* genotypes with higher seed weights may possess a higher amylase content and activity which contributes to a more rapid onset of germination and therefore to a stronger seed vigor.

To evaluate seedling vigor in rice, shoot dry weight is one agronomic character (Lu et al., 2007) besides others. Huang et al. (2017) found out, that the cultivar which was known to have a superior early vigor showed a higher partitioning of dry matter to the shoots and therefore higher leaf biomasses. This resulted in an improved photosynthesis. In the present study, the *japonica* cultivars Chomrong, X jigna and Yun Keng displayed clearly higher shoot DWs under optimal temperature conditions than the *indica* cultivars Faro 35 and IR 64. Aghaee et al. (2011) exposed, that low temperatures inhibited plant growth and led to a decrease in an overall plant biomass. Furthermore, he could demonstrate that cold tolerant cultivars showed an increased root/shoot ratio mainly due to a higher root dry matter. He concluded therefore, that a high root biomass is correlated with cold tolerance and a reduction in shoot biomass and plant height is an avoidance strategy. This hypothesis can only be supported partially by the present investigation. A decrease in plant biomass during the low temperature treatment was also observed in all cultivars tested in this study. This might be the result of limited water and nutrient uptake by the roots and also a decrease in net photosynthesis due to higher respiration losses. In contrast, all cultivars showed a decline in root DW and for most of the tested cultivars an increased shoot DW was detected when exposed to low temperatures. This occurrence is even clearly reinforced in *japonica* cultivars. This study therefore rather indicates that low temperature stress results in a lower root/shoot dry weight ratio which is even more decreased in *japonica* cultivars. Hence it can be assumed, that a higher shoot DW not only contributes to a higher seedling vigor under optimal conditions, but also under low temperature stress.

5.2 Effects of low temperature and cultivar on WMSR and SRUE

The results of the calculations of the weight of mobilized seed reserves (WMSR), seed reserve utilization efficiency (SRUE), as well as the seed reserve depletion percentage (SRDP) indicated, that the *japonica* cultivars, when exposed to optimal temperatures, mobilized on average higher weights of seed reserves, which resulted also in higher seedling DW. Part of the differences between *japonica* and *indica* cultivars can be ascribed to their higher kernel weight. Generally, there is a discrepancy concerning the advantage of a higher kernel weight or respectively larger seeds. As already mentioned earlier, Chen et al. (2015) and Lu et al. (2007) have been reported that the seed size and weight are associated with seed vigor in rice. Also, Bockous and Shroyer (1996) and Douglas et al. (1994) could provide experimental evidence for a positive correlation between seed size and seedling vigor for winter wheat. Roy et al. (1996) found out, that germination rate and seedling vigor index values of rice increased with the increase of seed size suggesting the selection of larger seeds for good stand establishment. On the other hand, Shroyer and Cox (1984) assumed that a higher seedling DW may not be a result of a larger seed size and a positive correlation between seed size and seedling DW has not been proved yet. Soltani et al. (2006) suspected that the oppositional reports may be because the seed reserve depletion percentage was not taken into account and that higher or lower seedling DWs are not based on a higher or lower seed reserve utilization efficiency. In other words, dry weights originated from cultivars with high seed weights but low seed reserve depletion percentage would be similar to that of cultivars with seeds lower in size and weight, but higher seed reserve depletion percentage. This hypothesis cannot be supported by the results of the present study. Only the seed reserve depletion percentage of X 265, with a seed weight of 23.43 mg (the lowest seed weight of all tested cultivars) and Yun Keng, with seed weight of 28.13 mg (the second highest), were significant higher than those of the other tested cultivars when exposed to optimal temperature conditions, while their seedling DW did not reach at all the dry weights of Chomrong for example with a seed weight of 31.99 mg and a much lower seed reserve depletion percentage. Hence, it can be assumed in the present study, that the higher seed weights of the *japonica* cultivars contributed to a higher seedling DW due to a higher mobilization efficiency when seedlings were exposed to optimal conditions. At low temperature treatment the differences in seedling DW were not as great as under controlled conditions, which is most likely due to increased respiration losses. Whereas the seed reserve mobilization was only delayed by about 13 days but the amount of seed reserves were almost as high as under optimal temperature conditions.

The seed reserve utilization efficiency was also higher for the *japonica* cultivars Chomrong and X jigna as compared to the *indica* cultivars Faro 35 and IR 64 at optimal temperature conditions. At low temperatures SRUE was decreased for the cultivars Chomrong, Faro 35, X jigna and Yun Keng which can be explained by higher respiratory losses during chilling stress. This is also confirmed by the results of Blum and Sinmena (1994) as well as the results of Kathun et al. (2015), who showed, that seed reserve utilization efficiency decreased as temperature is not favorable. A decline in seed reserve utilization efficiency at stress conditions were also reported by other researchers (Soltani et al., 2006; Sadeghi et al., 2011; Ansari et al., 2012).

On the other hand, the cultivars IR 64 and X 265 displayed an increase in SRUE and also developed more biomass in less time at unfavorable temperature conditions as compared to other cultivars, while their leaves exhibit obvious necroses towards the end of this experiment, which was one first indication for their cold sensitivity. Hence, cold sensitive cultivars seem to fight the cold by producing large quantities of biomass in a shorter time as compared to cold tolerant cultivars but they do not seem to possess the morphological adaption of the leaves to cold. However, to verify this hypothesis further investigations are needed on the morphological structure of the leaves of seedlings exposed to lower temperatures.

5.3 Effect of temperature and cultivar on seed reserve mobilization, plant growth and partitioning

Early seedling vigor is one of the major determinants for stable stand establishment of rice, especially in areas where direct-sowing is mainly implemented to reduce production costs.

In addition, environmental temperature is a very important external factor influencing the early seedling vigor of rice. Water and soil temperature fluctuate greatly during the sowing season in different areas or even in different cropping seasons in the same area. Thus, it is important to study diverse characteristic traits associated with early seedling vigor in multiple temperature environments and also consider possible genotype x environmental temperature interactions in order to select a suitable genotype for a given environmental temperature, sowing time or management strategy.

Many previous studies indicate that the *japonica* subspecies are generally more cold tolerant than *indica* (Lu et al., 2009; Zhang et al., 2012; Basuchaudhuri, 2014). This can be confirmed by the results of experiment I, if including MGT and T_{50} as a measure of evaluation. However, the phenotypic differences between the two subspecies, when exposing them to low temperatures of 20/12°C day/night, already marked differences in the survival rate, with the *japonica* cultivars Chomrong, X jigna and Yun Keng showing a better cold tolerance ability than the *indica* cultivars. Seedling leaves of IR 64 exhibited obvious necroses, while the *japonica* cultivars stayed completely intact. The other *indica* cultivar Faro 35 stayed mostly dormant throughout the entire period of experiment II and the only few (four out of 35) seedlings which emerged, displayed also visible necrotized leaves. Based on the low emergence rate of Faro 35, this cultivar could not be included in the assessment of experiment II. The cultivar X 265 could not be assigned to any unique subspecies on the basis of research. In matters of the phenotypic traits, X 265 developed only minor necroses on leaf tips. This and the intermediate behavior in regard to T_{50} and especially MGT encourages the assumption that the cultivar X 265 belongs to an intersubspecific *japonica* x *indica* cross line. This would also explain the different seed shape of X 265 (Fig. 4) which seems to be in-between the long and thin seed shape of the *indica* cultivars and the rather chubby shape of the *japonica* cultivars.

The fraction of material mobilized from the seeds at optimal temperature conditions was fairly similar among cultivars (0.62-0.74). Of this material, 0.55-0.71 was supposed to be used for plant growth. These values largely coincide with those of Asch et al. (1999), who reported mobilization efficiency values between 0.59-0.66 for five different upland rice cultivars. Yoshida (1981), who investigated the mobilization efficiency for lowland rice on the basis of growth experiments in the dark, also verified a mobilization efficiency of 0.60 and Labusch et al. (1989) demonstrated a mobilization efficiency value of 0.65 for winter wheat using carbon isotope tracer measurements.

At low temperatures on the other hand the fraction of mobilized seed material was reduced between 0.09-0.22 for all cultivars. This result does not coincide with the findings of experiment I of the present study where the weight of mobilized seed reserves of both temperature treatments was similar for each cultivar. Due to the number of available climate chambers, as well as the fixed time frame considered for the experiment, only seven different samples at different time intervals were managed. Therefore, no clear statement about whether the fraction of mobilized seed reserves is in fact reduced or is just delayed at lower temperature, can thus be made. This requires further studies with several samplings over a longer period of time.

Although mobilization efficiency was calculated amongst others by using the slope of the regression line of the decreasing kernel weight, a slightly lower final kernel weight would not have changed much in the calculation of the mobilization efficiency, so it can be assumed that this value is correct despite any deviations.

The mobilization efficiency was reduced at low temperature treatment for all cultivars. Hence, seedlings contribution to plant growth was smaller due to higher respiration losses as compared to seedlings growing at optimal temperature conditions, which was also reflected in seedlings dry weight (root, shoots and leaves). This finding is largely consistent with the findings of Hasan et al. (2004), who investigated the seed reserve mobilization efficiency of different wheat cultivars at temperatures of 15°C, 25°C and 35°C. At optimal temperatures of 25°C, seed reserve mobilization efficiency was significant higher compared to 15°C (low temperature stress) and 35°C (high temperature stress) in all wheat genotypes. A larger reduction in seed reserve mobilization efficiency at both unfavorable temperatures was experienced in heat sensitive genotypes. Hasan et al. (2004) therefore concluded that the lowest seed reserve mobilization efficiency at 15°C temperature was most likely because the respiration was not linked to a built-up of shoot and root biomass and could have led the thermal dissipation of the respiratory energy by way of cyanide resistant pathway or alternative oxidase pathway. The latter consists of one enzyme, the alternative oxidase (AOX), which is not linked to H^+ translocation, and thus ATP production, but has the potential to catalyze wasteful respiration in plant mitochondria. Abiotic stress such as low temperature stress induces the synthesis of AOX, which is thought to prohibit the production of reactive oxygen species (ROS) by helping to prevent over-production of ubiquinone-pool (Kurimoto et al., 2004). However, Kuk et al. (2003) proved, that an indicator for cold tolerant seedlings is a high respiratory homeostasis for maintaining shoot and root growth under low temperature conditions. In fact, in this study also a smaller discrepancy between the respiration losses of the two different temperature treatments of the cold tolerant *japonica* cultivars as compared to the *indica* cultivars were recorded.

Nonetheless, the present experimental design did not allow to statistically test cultivar x temperature interactions. Five seedlings sampled from the same growth chamber at the same time served as repetition in the present study and are therefore not considered to be "real repetitions". For this purpose, the entire experiment would have to be repeated in changing growth chambers at different times, which was not possible due to the number of available growth chambers in connection with the fixed timeframe set for this experiment but should be considered in the case of carrying out this or a similar trail.

The *japonica* genotypes Chomrong, Yun Keng and X jigna showed higher mobilization efficiencies at optimal temperatures as compared to the *indica* genotype IR 64 and the interspecific species X 265. Similar results were obtained by Asch et al. (1999), albeit not significant, the *japonica* genotypes WAB56-104 and Moroberekan displayed higher mobilization efficiencies as compared to the *indica* genotype Bouake 189, as well as the interspecific species V4 and the *O. glaberrima* species CG14. This result supports the hypothesis, that this might be due to the larger kernels of the *japonica* cultivars and thus a larger pool of available seed reserves. However, due to the small sample of cultivars an evaluation was not permitted and requires therefore further investigations.

In contrast to the findings of Asch et al. (1999), the *japonica* genotypes with significant higher kernel weights displayed a comparatively earlier onset of autotrophic growth as compared to the *indica* cultivar IR 64 at optimal temperature conditions. This result does not coincide with the idea that a late onset of the photo-autotrophic phase, is associated with higher kernel weights. In addition, due to the contradictory results of Asch et al. (1999), it cannot be assumed that a late or rather early onset of the autotrophic phase has anything to do with the genetic group (*japonica*).

However, at low temperatures with respect to the onset of the autotrophic phase, a contrary result has been demonstrated. Under these conditions, the *japonica* cultivars Chomrong, X jigna and Yun Keng showed a significant later onset of the photo-autotrophic phase as compared to the *indica* genotype IR 64. This suggests that a genotype x environment (G x E) interaction between the different genotypes exists and that each genetic group responds to temperature variation in regard to seed reserve mobilization and early seedling growth in a different way. As the interspecific species X 265 with the lowest seed weight displayed the same amount of days to the onset of the autotrophic phase as the *japonica* cultivars, also refutes the hypothesis that a late onset is associated with higher seed weights. It is conceivable that the late onset of the photo-autotrophic phase of the cold tolerant *japonica* genotypes, as well as the interspecific species X 265 is due to their morphological adaption to low temperatures by reducing their leaf area ratio, specific leaf area, as well as their relative growth rate. In this study the onset of the autotrophic phase was determined on the basis of the increasing weight gain of the seedling. It was therefore assumed, that the seedlings were not photosynthetic active before the plant became autotrophic but in fact, the majority of the seedlings had already green leaves even before their total seedling dry weight increased. The morphological adaption to low temperatures could therefore be an explanation of the delayed onset of the photoautotrophic phase of cold tolerant genotypes.

However, the time until the plant attainted their initial kernel weight again did not differ significantly among the cultivars at low temperatures. As a result, the time between the onset of the photo-autotrophic phase until the plant compensated their weight loss again was greatly extended for IR 64 as compared to the other cultivars tested in this experiment. This leads to the assumption, that either the photosynthesis decreased most likely due to a starch built-up in the chloroplasts, which then inhibited the photosynthetic enzymes (Sonoike, 1999), or IR 64 had not formed enough leaf material and the increase in total seedling dry weight was mainly caused by increased root development in conjunction with a slow seed reserve mobilization. This presumption is supported by the results obtained by calculating the root/plant dry weight ratio, as well as the leaf/plant weight ratio (Fig. 19). Accordingly, the root dry weight of IR 64 was clearly increased in relation to the total seedling dry weight, while the proportion of the leaf dry weight was significantly reduced in comparison to the *japonica* genotypes. This in reverse conclusion would mean, that cold tolerant cultivars increase their reduction in root development in favor of an increasing foliar production and show therefore great similarities to the root/plant dry weight ratio and the leaf/plant dry weight ratio as seedlings under optimal conditions. In addition, IR 64 mobilized a significant smaller fraction of material from the grain (0.49), which was supplementary mobilized much slower in comparison to cold tolerant cultivars X jigna or Yun Keng (Fig. 17, linear regression of kernel dry weight decrease). Hence, the total seedling dry weight decreased only slightly at the beginning and IR 64 had to produce merely small amounts of biomass in order to achieve a positive growth rate and thus, reached the photo-autotrophic phase earlier at low temperatures even though the respiration losses were marginal higher as compared to other cultivars tested in this study.

Comparing the rate in which the seed reserves were exhausted (Regression I) in both temperature treatments, it becomes obvious that the transition phase, i.e. the phase when photosynthesis already takes place but the reserve mobilization from the endosperm is still continuing, was greatly extended at low temperature treatment. In addition, temperature had a great effect on the growth rate throughout the time when seed reserve mobilization still took place. Hence, it can be concluded, that the enzymatic breakdown of the seed reserves must be greatly affected by temperature. This finding is also consistent with the observations from the germination experiment (Experiment I) and the results of Yoshida (1973) and Zakaria et al. (2001).

Considering the growth of the individual plant organs in the course of time, an almost complete suppression of root growth during the period when seedlings became photo-autotroph was observed (Fig. 18). This result is partly consistent with the findings of Asch et al. (1999) but unlike in his outcome, this timeframe does not coincide with the cessation of seed reserve mobilization (Fig.17). However, the short-term suppression of root growth supports the hypothesis of Asch et al. (1999) that this might be due to assimilate investment in the establishment of the photosynthetic apparatus.

A suppression in root growth during the onset of the autotrophic phase at low temperatures cannot be confirmed. To reveal whether the growth behavior of individual plant organs is in fact different at different temperatures or this was observed because the period between two sampling dates was chosen too long, further investigations are required.

6 CONCLUSION

It was shown in the present study that during germination and early seedling growth, seedlings of different rice subspecies differed strongly in their mean germination time, their mobilization efficiency of seed reserves as well as in the time they required to enter the photo-autotrophic phase at low temperatures of 20/12°C day/night, while they performed quite similar at optimal temperature conditions of 28/20°C day/night.

The *japonica* cultivars showed an overall better performance at low temperatures as compared to the *indica* cultivars. They germinated more rapidly, displayed no visual symptoms typical for cold damage and lost less seed reserves to respiration.

However, the *japonica* cultivars showed a delayed onset of the photo-autotrophic phase at low temperatures, which is under optimal conditions usually a trait not associated with early seedling vigor in rice (Asch et al., 1999) On the other hand, all cultivars needed approximately the same amount of time until the seedlings attained their initial kernel weight again. This indicates, that at low temperature conditions, not the onset of the photo-autotrophic phase is crucial in evaluating seedling vigor but probably the period of time between entering the autotrophic phase and the time until the seedlings compensated their initial kernel weight again. This proved to be depended on the pace of seed reserve mobilization, in conjunction with the amount of already formed photosynthetically active and still intact green plant organs.

Furthermore, it was shown that the seedlings which performed better under low temperatures, displayed a high shoot/root ratio and the root/plant ratio decreased during the period when the seedlings entered the photo-autotrophic phase in favor of an increased development of the photosynthetic apparatus.

In summary, the results of the previous study permit three general conclusions with respect to seed and early seedling vigor of rice cultivars under various temperature regimes: (i) temperature delayed the onset of germination but did not influence germination rate; (ii) *japonica* and *indica* genotypes showed different growing characteristics at different environmental temperatures and (iii) in order to select a suitable genotype for a particular environmental temperature different traits associated with early seedling vigor must be considered.

To confirm the results presented here it would be advisable to repeat the entire experiment several times with numerous samplings in different growth chambers to statistically evaluate genotype x environmental temperature interactions. A larger set of different genotypes would also be beneficial in order to provide breeders with a tool to remove cold sensitive genotypes from early breeding generations.

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X DECLARATION

I,

Elena Luyckx

Born on 03.07.1987 Matriculation number: 435895

hereby declare on my honors that the attached Master thesis has been independently prepared solely with the support of the listed literature references and that no information has been presented that has not been officially acknowledged.

Supervisor: **Prof. Dr. Folkard Asch**

Co-Supervisor: **Prof. Dr. Andreas Fangmeier**

Thesis topic: **Germination and early seedling partitioning of lowland rice in variable thermal conditions**

Semester: **7**

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