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in the Tropics and Subtropics (ARTS)**



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Institute of Plant Nutrition

Phyllochron and leaf development in field grown rice genotypes under  
varying thermal environments of a high altitude cropping system

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## Dedication

*To my parents*

*Jaga-Kumbha Khanal*

*For their consistent Support,*

*Encouragement and Love*

## Table of contents

1	Introduction .....	10
1.1	Background.....	10
2	Hypothesis and Objectives .....	14
3	Literature review .....	15
3.1	Rice-wheat crop rotation in Nepal.....	15
3.2	Rice phenology .....	16
3.3	Phyllochron and leaf development in rice .....	17
3.4	Effect of temperature on rice leaf appearance.....	18
4	Materials and Methods .....	19
4.1	Genetic materials.....	19
4.2	Location and treatments of the experiment.....	19
4.3	Layout of the field .....	21
4.4	Phenological observations and interpretation.....	22
4.5	Statistical analysis .....	23
5	Results.....	24
5.1	Temperature, photoperiod and rainfall .....	24
5.2	Effect of planting dates on different leaves.....	25
5.2.1	Appearance and number of leaves on main culm.....	25
5.2.2	Leaf appearance pattern at different planting dates .....	26
5.2.3	Leaf duration at different planting dates .....	27
5.3	Temperature effects on the appearance rate of different leaves .....	28
5.3.1	Temperature effect on the appearance of the first leaf.....	28
5.3.2	Temperature effects on the appearance rate of leaves two to seven .....	29
5.4	Leaf Development and Senescence .....	31
5.4.1	Leaf development stages at different planting dates .....	31
5.4.2	Canopy senescence level across the age of rice plant.....	37
5.5	Physiological phases of different leaves.....	37
6	Discussion .....	40
6.1	Effect of planting dates on different leaves.....	42
6.2	Effect of temperature on different leaves.....	43
6.3	Leaf development and senescence .....	44
6.4	Physiological phases of different leaves.....	45
7	Conclusion .....	46
8	References .....	47
	Appendix.....	51

## Abstract

Rice-wheat crop rotation in Nepal includes a pronounced season between the wheat and the rice during which a large share of soil borne nutrients may be lost from the system if not properly managed. To make use of early nutrient and water availability this transitions seasons can either be shortened or extended to allowing a third crop to be grown, by changing the planting date of the rice which may influence its crop duration. Adaptation of the cropping calendar would require rice genotypes adapted to the new growing environment. One factor influencing crop duration is the speed of crop establishment i.e. vegetative development after sowing, which is known to be influenced by the thermal environment. The objectives of this study were: to examine leaf appearance patterns and leaf duration for a number of genotypes planted at different dates, relate this to the thermal environment and identify key characteristics for crop establishment needed to be present in a genotype planted at dates different from the recommended one. Six contrasting cultivars were sown at 8 dates in 15 day intervals starting 24<sup>th</sup> April 2004 at the experimental field of Regional Agriculture Research Station, Lumle, Nepal. Data were recorded daily for individual leaf appearance and their development on the main culm. Planting date influenced leaf appearance rate (LAR) decreasing the total number of leaves on the main culm at later planting dates. First LAR was positively linearly correlated with temperature, whereas the LAR of leaves 2-4 showed a quadratic response to mean air temperature with a distinct genotypic optimum. Later LAR were not influenced by temperature. Later appearing leaves stayed longer physiologically active, particularly at later planting dates, indicating other control mechanisms for leaf development than temperature. Among the genotypes tested Khumal-4 showed increased leaf durations already at early planting dates, rendering this genotype a potential candidate for early sowing dates due to its increased LAR and low optimum temperature. To validate this choice, however, an assessment of the reproductive performance of this genotype is needed and recommended.

## List of Figures

Figure 1. Map of Nepal .....	199
Figure 2. Hand weed of the experimental plots.....	211
Figure 3. Layout of the experimental field.....	211
Figure 4. soaking of seeds before sowing .....	22
Figure 5. Dibbling of the sprouted seeds .....	22
Figure 6. Experimental field from planting one to eight.....	23
Figure 7. Marking of different leaves with colored pen.....	23
Figure 8. Climatic pattern of the experimental site in 2004, Lumle, Nepal. ....	244
Figure 9. Number of leaves on the main culm over time across the different planting dates.....	255
Figure 10. Dates of leaf appearance as a function of planting date .....	266
Figure 11. Leaf duration after appearance of six rice cultivars across four planting dates.....	277
Figure 12. Development rate of the first leaf, as a function of mean air temperature. ....	29
Figure 13. Appearance rate of 2 <sup>nd</sup> , 3 <sup>rd</sup> & 4 <sup>th</sup> leaf as a function of mean air temperature .....	30
Figure 14. Appearance rates of different leaves (reciprocal of duration) as a function of mean air temperature for two rice cultivars.....	30
Figure 15. Development stages of leaf 1 on the main culm .....	31
Figure 16. Development stages of different leaves of rice cultivar IR31785 at different planting dates. ....	32
Figure 17. Development stages of different leaves of rice cultivar Sabitri at different planting dates. ....	33
Figure 18. Development stages of different leaves of six different rice cultivars at 1 <sup>st</sup> planting date.....	33
Figure 19. Development stages of different leaves of six different rice cultivars at 2 <sup>nd</sup> planting date.....	34
Figure 20. Development stages of different leaves of six different rice cultivars at 3 <sup>rd</sup> planting date.....	34
Figure 21. Development stages of different leaves of six different rice cultivars at 4 <sup>th</sup> planting date.....	35
Figure 22. Development stages of different leaves of six different rice cultivars at 5 <sup>th</sup> planting date.....	35
Figure 23. Development stages of different leaves of six different rice cultivars at 6 <sup>th</sup> planting date.....	36
Figure 24. Development stages of different leaves of six different rice cultivars at 7 <sup>th</sup> planting date.....	36
Figure 25. Relationship between canopy senescence level and rice plant age at leaf initiation. ....	37
Figure 26. Rice leaf duration in relation to different physiological phases .....	38
Figure 27. Diagram showing rice-wheat crop rotation and possible changes in cropping calendar.....	40

## List of Tables

Table 1 Classification of the Test Materials by Duration Type, Country of Origin, and Presence in Nepal (N). 20

Table 2 Weekly maximum, mean and minimum temperatures (°C) observed during the research period at the experimental site (18th April to 31st December 2004)

51

## 1 Introduction

### 1.1 Background

Rice is the staple food crop for more than 400 million people in the world out of which more than 15 million people depend on rice based cropping systems in Nepal. Nepal's most important crop production system is the rice-wheat rotation. Rice is the most important food crop of Nepal and is cultivated under a wide range of ecological conditions. Nepal is an agricultural country with more than 76% of the population depending on agriculture for their livelihood. Agriculture contributes 38 % to the national gross domestic product. Rice is grown from elevations of 60 masl (above sea level) up to 3050 masl, which is the highest altitude for rice cultivation in the world. The average yield of rice is 2.7 tha<sup>-1</sup> in Nepal (FAO 2003). Wheat is grown after rice in terms of area and production. An increase in yield of individual crops and cropping intensity is urgently needed to cope with the increasing population growth.

Rice-wheat rotation is characterized by a more or less pronounced dry to wet transition period after harvesting wheat and before transplanting rice. Proper management of this transition period leads to nutrient conservation, higher nutrient use efficiency and increased sustainability of the system (Pande and Becker 2003). One possible option to use the fallow period is to plant a transition season crop, but this will most probably lead to a shift the planting date for rice, particularly in high altitude systems where the transition period is short. There are potential transition season crops that can be grown during the dry to wet transition period. Most of them are legumes hence enhancing the nutritional status of soil if ploughed back after harvest. These crops can be grown as grain crops like Kidney beans, Cowpeas or as green manure crops like Mucuna or Vigna. These transition season crops have 90 to 110 days crop duration. Hence, planting of these crops may shift the recommended planting for the rice crop. Since rice phenology depends on the photo-thermal environments, changing the planting date may shift thermo-sensitive phenological stages of the rice crop into thermally critical environments which could lead to a prolonged duration, a delay in flowering

and may severely reduce yields due to poor source or sink formation (Dingkuhn *et al.*, 1999).

Two types of rice, *japonica* and *indica*, are growing in Nepal. *Japonica* types are long duration cultivars whereas *indica* types have short crop duration. Crop duration varies from 118 to 174 days after transplanting depending on the variety and their growing areas. Chilling injury in rice is common in Nepal in high altitude areas (i.e. >1000 masl). Of 1.4 million hectares of rice in Nepal, 26% are grown in temperate areas (1000-2000 m) (Sthapit *et al.*, 1996), and spikelet sterility caused by chilling is a major constraint above 1500 m, limiting both the area of production and the length of the growing season (Sthapit and Shrestha, 1991).

Understanding rice crop phenology is important to develop better adopted calendars for a particular agro-ecological system since rice phenology is influenced by the photo-thermal environment. Hence, rice cultivars transplanted or sown at different dates may have variable crop duration. Variable crop duration and temperature stresses, however, limit yields and calendar options (Dingkuhn, 1994). Accurate timing of crop growth duration is of great importance in high altitude cropping systems to avoid spikelet sterility due to chilling injury. Crop duration is influenced by (1) the thermal time a particular genotype needs to accumulate before flowering can be induced (BVP), (2) on the photoperiod sensitivity of that genotype (PSP), and (3) on the speed of crop establishment after sowing (source build-up). Indicators suited to estimate crop establishment would be leaf plastochron and phyllochron in relation to their temperature sensitivity.

Phyllochron is the time interval between the emergence of one leaf and the next. The length of phyllochron is determined particularly by temperature, but it is also affected by day length, humidity, soil quality, exposure to light and air, and nutrient availability. Rice shows a striking feature that the phyllochron is synchronized with the plastochron (Itoh *et al.*, 2001). . Hence, it is important to know the leaf initiation in rice. The time interval between successive leaf initiations is called plastochron (Langer, 1972 in Counce *et al.*, 2000). The

problem with determining the plastochron is that the initiated leaves are not visible with the bare eye.

Leaf appearance is one of the important aspects of development in rice (Gao *et al.*, 1992). Rice leaf development and physiological phases are important in relation to photosynthesis and yield. Leaf initiation in rice begins just after seed soaking. It takes some time after the initiation to the appearance of a leaf. Each rice tiller produces 10-18 leaves in its life (Yoshida, 1981). The last leaf in rice to appear is called the flag leaf, which appears after panicle initiation. Thus, for each leaf a minimum of three physiological phases can be defined (1) initiation to almost full extension – the leaf is a positive (productive) sink for carbohydrates (2) the physiological active period between almost full extension to about 25% senescence – the leaf is a source, and (3) the senescence period between 25% senescence and leaf death – the leaf is a negative sink (Dahal, 2004; Das, 2004)

Leaf initiation, appearance and development are influenced by temperature. Most studies related to leaf phyllochron and leaf development in rice were conducted in greenhouses and tropical environments. Hence, there is lack of research work for high altitude rice systems. In addition to this, farmers of the research site followed a rice-wheat crop rotation since a long time and there is need of transition season crop after the harvest of wheat and before the transplanting of rice to improve the transition period. For the use of this transition period, there is need to evaluate a large number of rice genotypes under different planting dates.

Based on the idea of a more flexible cropping calendar to support a transition crop, the problem that the wheat member in the rotation cannot be shifted, and the possibility to shift the rice rotation member either to an earlier or to a later planting date in order to accommodate nutrient conserving measures or a transition crop, this research project was focussed on the early establishment of the crop to answer the following research questions:

1. What is the effect of change in planting dates on leaf appearance pattern and leaf duration on different rice genotypes?

2. What is the appearance rate of different leaves under varying temperature?
3. What is the effect of different planting dates on leaf development stages on different rice genotypes?
4. Does the physiological potential of individual leaf change at different planting dates.

## 2 Hypothesis and Objectives

The underlying hypothesis of this study is that rice cultivars response differently with varying thermal conditions. Hence, based on the above research questions the following hypotheses are the basis for the objectives.

1. Changes in planting date lead to different leaf appearance pattern and leaf duration.
2. Leaf appearance rate is influenced by temperature.
3. Changes in planting date lead to differences in leaf development stages.
4. Each leaf has different physiological phases at different planting dates.

Based on above hypotheses the following objectives were formulated for this research work:

1. To examine the effect of planting dates on leaf appearance pattern and leaf duration.
2. To assess the effect of variable temperature on leaf appearance.
3. To describe the leaf development stages under different planting dates.
4. To examine the physiological phases of different leaves at different planting dates.

### 3 Literature review

#### 3.1 Rice-wheat crop rotation in Nepal

Rice is one of the world's most important crops (Dawe, 2000). It is the staple food of the majority of the Asian population, which has grown for about 1.70 billion in 1961 to 3.52 billion in 1997 (IRRI, 2003). Rice-wheat production systems occupy 24 million hectares of cultivated land in the Asian Subtropics. In south Asia, the system occupies about 13.5 million hectares and provides food for 400 million people (Ladha *et al.*, 2000). The emergence of the rice-wheat system in south Asia serves as an important source of food supply and is the result of the green revolution in wheat and rice (Pingali, 2001). Hence, the rice-wheat system is one of the most important cropping systems for food self security in the region. This system is found in the fertile, hot semi-arid to hot sub-humid regions of the Indus and Gangetic alluvial plains of Bangladesh, India, Nepal and Pakistan (Hobbs and Gupta, 2001). In this system rice is grown in the warm, sub-humid monsoon, summer months and wheat in the cooler, drier, winter season. The rice-wheat cropping system is practiced in about 0.6 million hectares in Nepal, extending from the Terai to sub-temperate mid-hill region up to the altitude of 4000m. Growth in food grain production in Nepal has been unable to keep pace with increasing demand for food. Farmers will have to produce more food from the same area, as the further expansion of cultivated area is limited. The problem could be solved to some extent by increasing cropping intensity (Regmi, 1997). One means of increasing cropping intensity would be to adopt a triple cropping system such as rice-wheat-mung bean or rice-wheat-rice in the Terai and in the lower river basins in the hills, where permanent irrigation facilities are available. The decline in yield of the rice-wheat system in both farmers and on station field experiments has been identified (Abrol *et al.*, 2000; Hobbs and Morris, 1996). The long term experiment in Bhairahawa, Nepal, also showed a similar decline in grain yield of early rice during 13 years (Regmi *et al.*, 2002). Mansky *et al.*, (2000) reported low and stagnating yields of rice and wheat over the past two decades. There is need of sustainable practice to increase the yield and sustain the system. Pande and Becker (2003) reported

on increased crop yield and improved soil fertility status after growing transition season crops in the Terai.

### 3.2 Rice phenology

Phenology is the study of plant growth and development with respect to the timing of various growth stages, i.e, flowering, fruit development and senescence. Accurate prediction of crop phenology is important for modeling and crop improvement and management actions (Yin, 1996). Rice phenology is influenced by the photo-thermal environment. Sie *et al.*, (1998) reported variable crop duration of rice genotypes when transplanted at different planting dates. Crops require a specific amount of heat to develop from one point in their life-cycle to another, such as from seeding to the four-leaf stage. Crop duration can be calculated using the concept of growing degree days (GDD). Growing degree days (thermal time) is a way of assigning a heat value to each day. The values are added up to give an estimate of the amount of seasonal growth of a crop. It has been observed in many crops that the rate of development is linearly related to the daily mean temperature above a base temperature up to an optimum temperature, beyond which the rate decreases, again linearly, until a maximum temperature is reached. For temperatures below the base temperature or above the maximum temperature, the rate of development is zero. Three “cardinal” temperatures can therefore be identified: base temperature ( $T_{base}$ ,  $T_{BD}$ ; °C), optimum temperature ( $T_{opt}$ ,  $T_{OD}$ ; °C), and maximum temperature ( $T_{high}$ ,  $T_{MD}$ ; °C). For rice, these values are typically 8, 30, and 42 °C, respectively (Gao *et al.*, 1992); however, those values may vary considerably between genotypes (Dingkuhn *et al.*, 1994). A specific growth stage (e.g., flowering) is reached when a certain number of degree-days ( $T_{sum}$ ) has been accumulated. Rice phenological phases can be divided in to four phases following a simple model used at IRRI:

1. The basic vegetative phase (BVP), from emergence to the start of the photoperiod-sensitive phase.
2. Photoperiod-sensitive phase (PSP), from the end of the basic vegetative phase to panicle initiation.

3. Panicle formation phase (PFP), from panicle initiation to (50%) flowering.
4. Grain-filling phase (GFP), from (50%) flowering to physiological maturity.

### 3.3 Phyllochron and leaf development in rice

Phyllochron is the period of time between the emergence of one phytomer (a set of tiller, leaf and root which emerges from the base of the plant) and emergence of next. Phyllochron length reflects the speed of the rice plants "biological clock". The thermal interval for leaf appearance is a critical variable for modeling plant development and growth (Birch *et al.*, 1998). Phyllochron varies across environments, but is generally constant for a species grown in specific environments. The phyllochron in maize is lower in temperate environments than in tropical and subtropical environments (Birch *et al.*, 1998). In germinating rice the sequence of initial events is as follows: A prophyll (rudimentary leaf) emerges from the coleoptile. This is followed by the emergence of the first leaf above the prophyll. A typical rice leaf consists of sheath, blade, ligula and auricles.

The number of leaves varies with the cultivar. Short duration and photoperiod insensitive varieties have fewer leaves than long duration and photoperiod sensitive varieties. The first few leaves are smaller in size and develop faster than the later stage leaves. According to Yoshida (1981) most early to medium duration rice cultivars produce 10 to 18 leaves on the main culm, and the total leaf number of photoperiod insensitive cultivars is roughly constant even under variable photo-thermal conditions. In contrast, Sie *et al.*, (1998) observed variable number of leaves with different planting dates, which was correlated to crop duration. They found that early leaf generations (i.e., L1 to L4) appeared in more rapid succession than later-appearing leaves. Yoshida (1981) has also reported an average duration between successive leaf appearances of 4 to 5 days before panicle initiation and 7-8 days afterwards. The rate at which plants produce leaves is important for canopy development, since leaves are the source of carbohydrates for the plant.

### 3.4 Effect of temperature on rice leaf appearance

Temperature controls the rate of leaf appearance (Ritchie and NeSmith, 1991 in Birch *et al.*, 1998). Leaf appearance rate can be used to determine the response of temperature to crop development (Ritchie and NeSmith, 1991 in Yin and Kropff, 1996). Temperature is the primary factor controlling phenological development rates, with photoperiod and vernalization often being important for some crops as well (McMaster, 1997). Baker *et al.*, (1990) reported a sudden decrease in leaf appearance rate (LAR) at the transition from vegetative to reproductive growth stage. In addition, Sie *et al.*, (1998) reported that rate of germination and leaf appearance rate for the first four leaves depend on temperature. Sie *et al.*, (1998) reported that temperature was the main determinant of variable crop duration; longer durations were generally associated with increased leaf numbers. While, Lee (2001) reported under a range of temperatures (15 °C to 27 °C) the final number of leaves on the main culm was constant as 15 regardless of temperature in rice. Sie *et al.*, (1998) mentioned that leaf development rate (which is reciprocal of days to emergence) is linearly correlated with mean water temperature. They further reported that the appearance rate of first to the fourth leaf of different rice genotypes followed a parabolic temperature response with a distinct optimum temperature. Das (2004) developed a system using temperature independent canopy senescence levels as determinants for leaf initiation at later growth stages in rice that was developed as a consequence of results showing differences in leaf appearance rates among salinity treatments in the same thermal environment.

## 4 Materials and Methods

### 4.1 Genetic materials

Thirty-two rice cultivars were selected for phenological analysis, with the objective to cover the genetic diversity of rice currently cultivated in different agro-ecosystems (Table 1). Nineteen cultivars from Nepal and thirteen reference cultivars from international germplasm collections were used. The international reference cultivars were used to know their phenological responses in high altitude conditions and suitability at that site. The cultivars IR4630-22-2-2 and IR31785-58-1-2-3-3 were included because they had been already characterized for their leaf appearance patterns under greenhouse and field conditions.

### 4.2 Location and treatments of the experiment

A phenological experiment based on eight sowing dates with 15-day intervals, was conducted at the Regional Agriculture Research Station (RARS)'s experimental farm at Lumle (elevation-1740 m, latitude- 28 18' N, longitude 83 48' E) Kaski, Nepal.

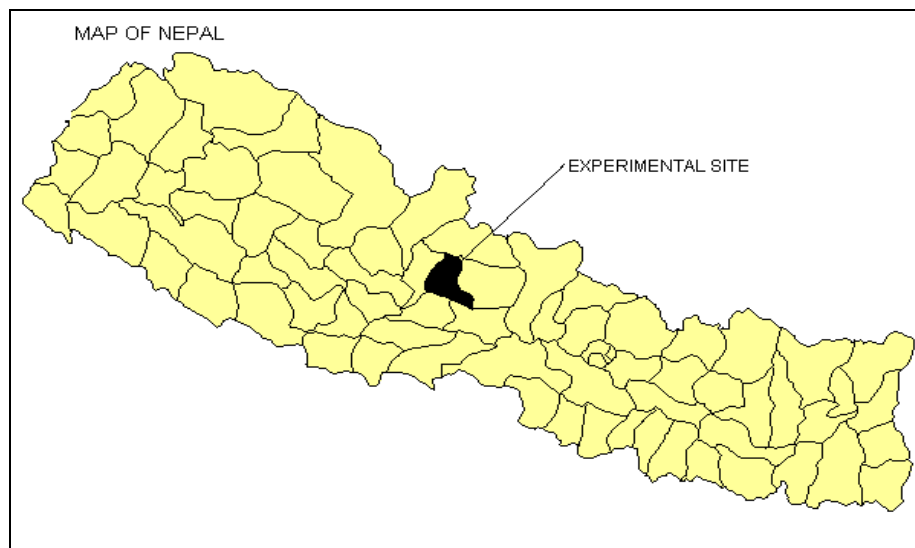


Figure 1. Map of Nepal

Table 1 Classification of the Test Materials by Duration Type, Country of Origin, and Presence in Nepal (N).

Genotype No		Duration	Origin	N
1	IR28	short	Philippines	
2	IR64	short	Philippines	
3	IR4630-22-2	medium	Philippines	
4	IR31785-58-1-2-3-3	short	Philippines	
5	I KONG PAO	short	Taiwan	
6	JAYA	medium	India	N
7	WAS30-11-1-4-6-1-2			
8	WAS30-11-1-4-6-1-1-3			
9	ITA306			
10	WAB450-24-3-2-P18-HB			
11	CG14			
12	32XUAN 5C			
13	SAHEL108			
14	MANJUSHREE-2	long	Nepal	N
15	JETHOBUDO	long	Nepal	N
16	HIMALI	medium	Philippines	N
17	KANCHAN	medium	Philippines	N
18	CHAINUNG-242	medium	Taiwan	N
19	KHUMAL-2	medium	Nepal	N
20	KHUMAL-4	medium	Nepal	N
21	KHUMAL-6			N
22	KHUMAL-9	medium	Nepal	N
23	KHUMAL-11	medium		N
24	HARDINATH-1	short		N
25	SABITRI	medium	Philippines	N
26	MASULI	medium	Malaysia	N
27	MAKAWANPUR-1	medium	Shri lanka	N
28	CHAITE-2	short	Philippines	N
29	CHAITE-4	short	Philippines	N
30	CHAITE-6	short	Philippines	N
31	MACHAPUCHRE-3	long	Nepal	N
32	CHOMRONG	long	Nepal	N

The study area falls within the sub-tropical summer rain agro-ecological zone with cool dry winters and warm humid summers. The total annual rainfall of the area is about 5000 mm. Much of the rainfall concentrated in the monsoon season from April to August. The mean maximum temperature from April to December was 15.5 °C to 23.5 °C. The experimental soil was a well drained acidic sandy loan soil.

Treatment	Soaking dates	Treatment	Soaking dates
D1	24-Apr-04	D5	23-Jun-04
D2	9-May-04	D6	8-Jul-04
D3	24-May-04	D7	23-Jul-04
D4	8-Jun-04	D8	7-Aug-04

The plots were received a basal application of NPK and N - top dressing with urea. Hand weeding was done as required.



Figure 2. Hand weeding of the experimental plots.

#### 4.3 Layout of the field

Genotypes were organized by planting date in a fully randomized block design with a plot size of 1 square meter. Between the plots, there was enough space to move easily without damaging the plants. All eight planting dates were on neighboring plots.



Figure 3. Layout of the experimental field

Water supply was mainly rainfall with additional irrigation by irrigation pipe. Each plot was clearly labeled with variety, planting date and number. Seed was pre-soaked for 24 hours and kept in a germination chamber for 48 hrs.

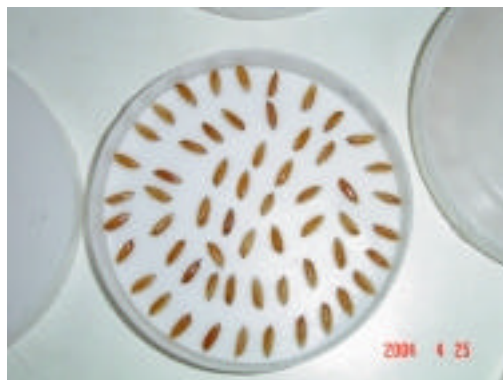


Figure 4. soaking of seeds before sowing

Only sprouted seeds were sown. Ninety eight seeds were dibble-seeded into 49 pockets of equal distance (two seeds per pocket). The outer row of seedlings was served as a border row and used to determine panicle initiation. Four hills of the inner sample square were used for leaf development observations.



Figure 5. Dibbling of the sprouted seeds

#### 4.4 Phenological observations and interpretation

Seedling emergence, first tillering, panicle initiation, booting, heading, 50% flowering, 100% flowering and maturity were visually assessed on a daily basis on all plots though out the experiment. Observations were made in each plot on the middle 25 hills and all data were recorded.



Figure 6. Experimental field from planting one to eight

Leaf appearance, leaf development and leaf duration were monitored on the main culm of selected contrasting genotypes (6 cultivars). For the leaf development length, percent unfolded, extension, percent senescence and death were recorded as millimeters and dates. A leaf position was identified by appearance and by marking them with different colored pens.



Figure 7. Marking of different leaves with colored pen

Daily minimum and maximum temperature, rainfall and photoperiod data were obtained from the on-site meteorological station.

#### 4.5 Statistical analysis

Data obtained from the field was entered into Excel spreadsheets and statistically analyzed with SPSS and sigma plot.

## 5 Results

### 5.1 Temperature, photoperiod and rainfall

The climatic data for this experiment were collected from a meteorological station located at the site. During the year, daily maximum, mean and minimum temperature varied between 27 °C and 12 °C, 6.3 °C and 22.5 °C, and 0 °C and 19.5 °C respectively. During the year, photoperiod varied from 10.20 h to 13.8 h.

During the experimental period, maximum temperature varied between 27°C and 22.5 °C and minimum temperature ranged from 8 to 19.5 °C. Daily mean temperature varied between 15.5 °C to 22.5 °C. Average daily temperatures increases with later planting dates. Photoperiod varied by 3.6 h., with a maximum of 13.80 h for calendar day 173 and a minimum of 10.20 h for calendar day 357 (Figure 8). Rainfall of the area was concentrated between calendar day 94 and 287.

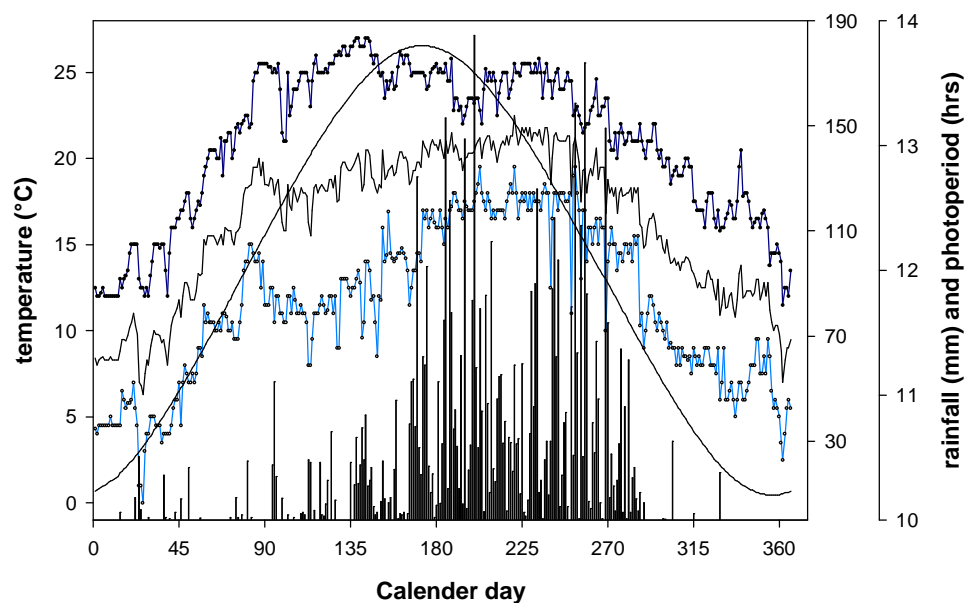


Figure 8. Annual pattern of photo-period, rainfall and daily minimum, mean and maximum air temperatures during field experiment in 2004, Lumle, Nepal.

## 5.2 Effect of planting dates on different leaves

### 5.2.1 Appearance and number of leaves on main culm

The appearance of leaves was recorded daily in the field. When considering the cumulated number of leaves on the main culm at each observation date, three leaves were noted after 20 days, 6 leaves after 35 days, and the appearance of other leaves were significantly different among planting dates after 40 days. There was change in total number of leaves on main culm over time. For example, cultivar Khumal-4 (local cultivar for midhills) produced 12 leaves, 11 leaves and 10 leaves after 75 days at planting date 2<sup>nd</sup>, 5<sup>th</sup> and 7<sup>th</sup> respectively (Figure 9). Figure 9 shows the number of leaves on the main culm increased linearly over time for 45- 50 day after seed soaking (i.e. up to the 7-8 leaf). The phyllochron (time interval between two successive leaves) showed a change in time interval between leaves depending upon plant age and also across planting dates. The average duration between successive leaf appearances across the eight sowing dates (cultivar IR 31785) was about 4.3 days for leaves 1 to 4, and about 8.8 days for leaves 7 to 12.

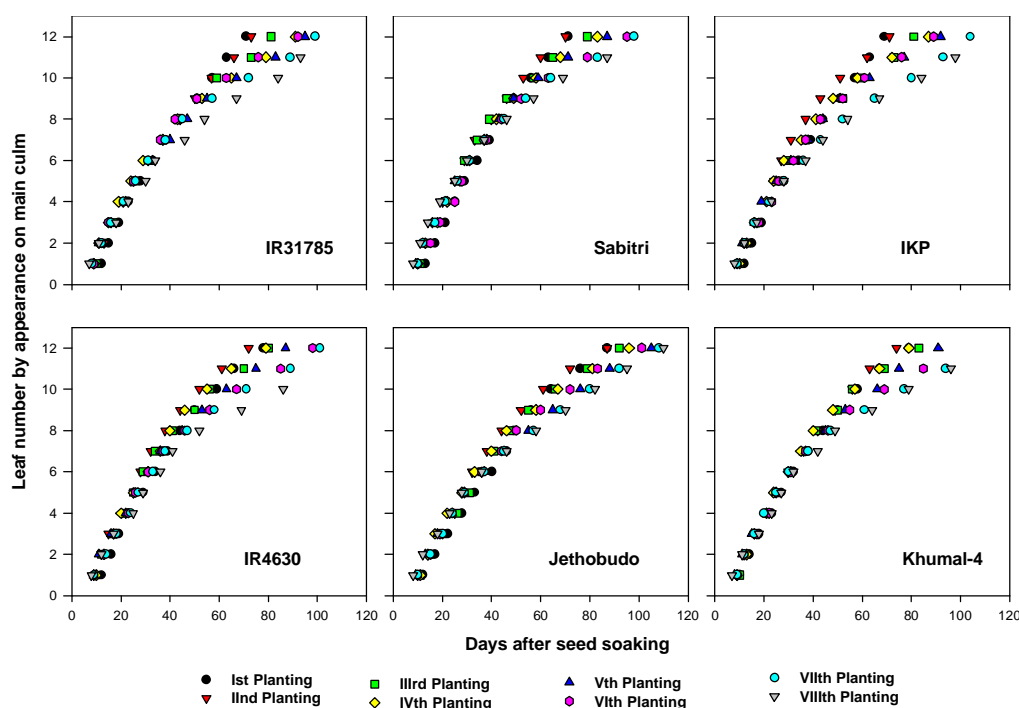


Figure 9. Changes over time (number of days after seed soaking) in the number of leaves on the main culm across the different planting dates.

### 5.2.2 Leaf appearance pattern at different planting dates

Figure 10 shows the sequential dates of appearance of individual leaves across eight planting dates. The same figure shows differences among cultivars in days needed to leaf appearance. Local cultivar Khumal-4 appeared 1-2 days earlier than other cultivars in experiment. In general, the first five leaves appeared within 30 days after soaking in all cultivars and in all planting dates. Leaf positions greater than 5 appeared with increasing intervals in all cultivars; however, cultivars differed strongly in the number of days leaf appearance was delayed. With increasing planting date the delay in leaf appearance of leaf position greater than 5 increased significantly in all cultivars. For example, cultivar I Kong Pao (IKP) shows that leaf number 11 appeared 63 days after seed soaking in first planting whereas the same leaf appeared 98 days after seed soaking in the eighth planting date. Cultivar Khumal-4 (local cultivar for midhills) shows that leaf number 11 appeared 67 days after seed soaking in first planting whereas the same leaf appeared 96 days after seed soaking in the eighth planting. Hence, late planting delayed the appearance of leaves in that environmental condition.

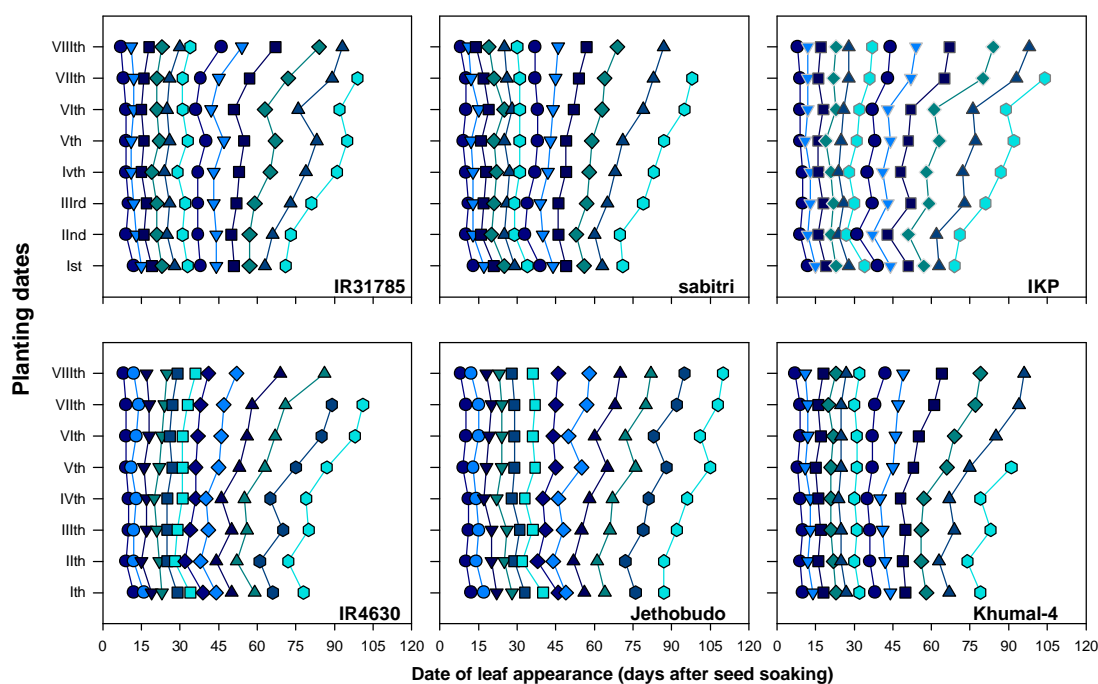


Figure 10. Dates of appearance of subsequent leaves (1-12) as a function of planting date (date of seed soaking) six rice cultivars planted at 15 days intervals.

### 5.2.3 Leaf duration at different planting dates

Leaf duration was calculated by subtracting leaf appearance date from the date of death of a particular leaf. Leaf duration increased as the leaf number progressed. Figure 11 shows that a particular leaf has different duration at different planting dates. However, the differences were not clear in the earlier leaves. The leaf duration increased at later planting dates for later leaves. In Figure 11, the leaf duration of leaf number 9 (cultivar IR4630) increased from 67 days at first planting, to 80 days at third planting, and to 110 days at fifth planting. This increase in duration was common among cultivars after leaf 7. The leaf duration increased significantly for later leaves at planting date fifth and seventh. Mean air temperature decreased during the later part of experiment hence, the overall duration of the leaf may have been influenced by temperature.

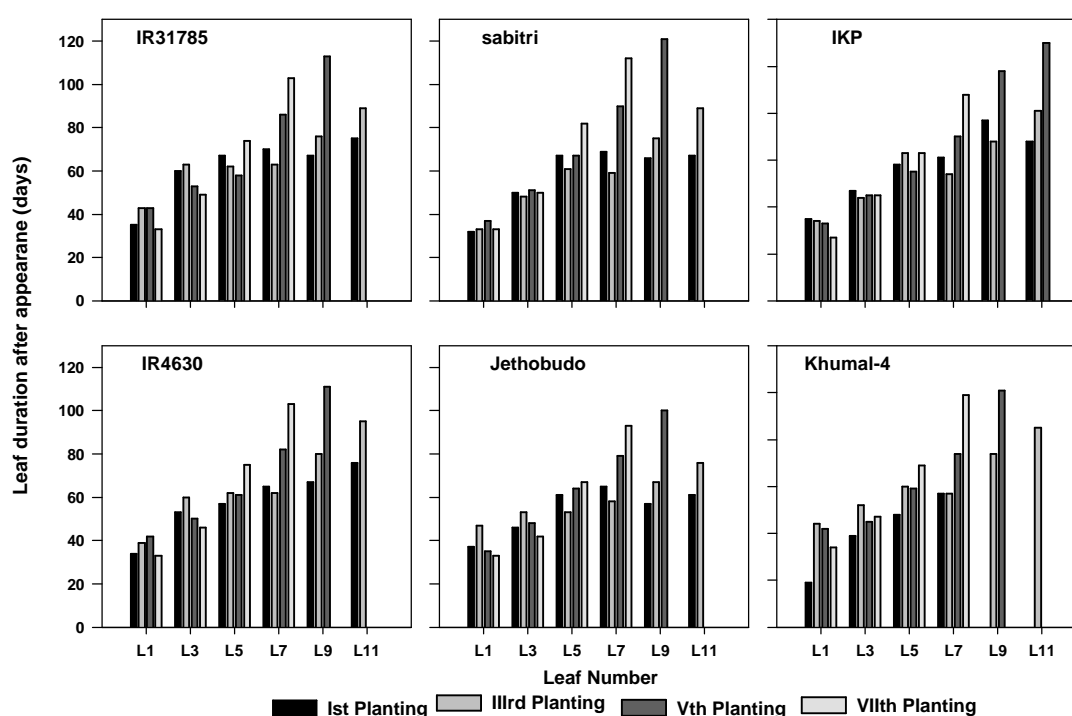


Figure 11. Duration of selected leaves (after appearance) of six rice cultivars shown exemplarily for four planting dates.

### 5.3 Temperature effects on the appearance rate of different leaves

Temperature effects on leaf appearance rates were studied for a number of successive leaves. When leaf appearance rate was regressed against the average mean air temperature for the period between two successive leaf appearances, the appearance of the first leaf needed to be calculated in a different way, since its appearance completes the germination process and is not preceded by the appearance of another leaf.

#### 5.3.1 Temperature effect on the appearance of the first leaf

The appearance rates (reciprocal of days to appear) of first leaf were regressed across the mean air temperature from the seed sowing to the appearance of first leaf for eight planting dates. Figure 12 shows a positive linear correlation between the appearance rates of the first leaves and mean air temperature (variety IR31785  $r^2= 0.82$ ,  $p<0.05$ ). Increasing mean air temperature reduced the number of days needed for the first leaf to appear. This relation was common for all six rice genotypes. The conditions of the fourth planting date somehow failed to produce the same relationship. This may be due to the high fluctuation in temperature during that planting date. These data were marked as outliers in the plots by a square symbol and were excluded from the regression analysis.

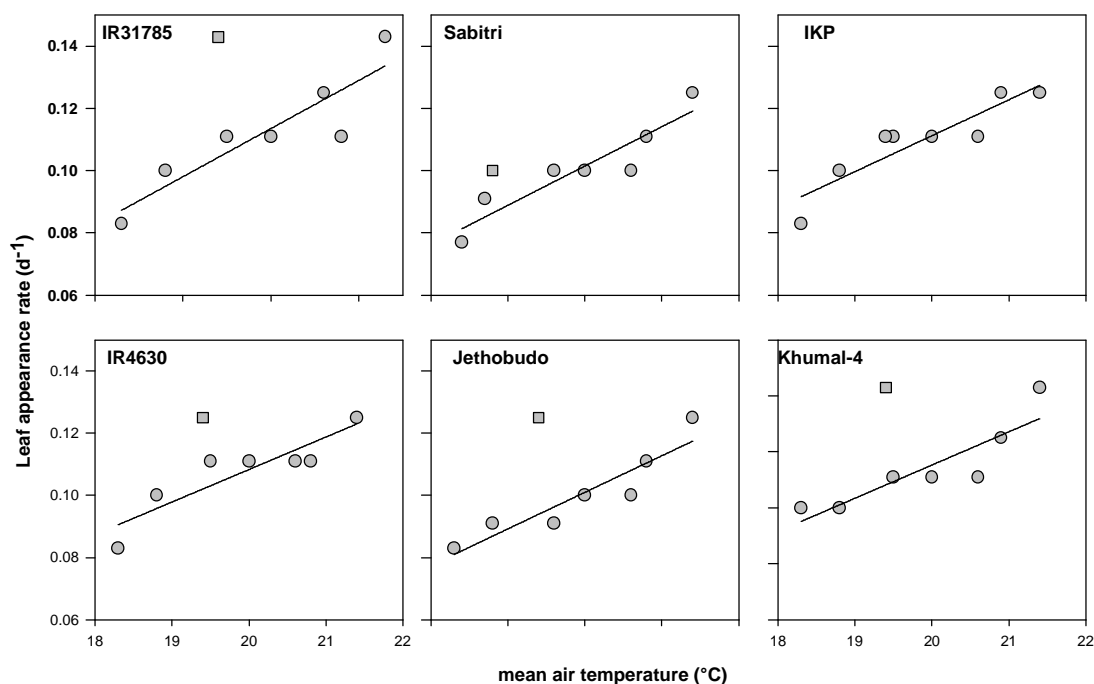


Figure 12. Development rate from seed soaking to the appearance of the first leaf, as a function of mean air temperature between sowing and the appearance of the leaf.

### 5.3.2 Temperature effects on the appearance rate of leaves two to seven

Plotting the average mean air temperature between two leaf appearances for any leaf appearance other than the first leaf against leaf appearance rates for several planting dates resulted in clearly peaked curves with a distinct maximum. The appearance rate of the second to the fourth leaves followed a quadratic temperature response with a distinct  $T_{opt}$ , below and above the value there was pronounced decrease in leaf appearance rate. Although the patterns were not clear in all six cultivars, some cultivars differed markedly. Figure 13 shows Cultivar IR31785 had a distinct  $T_{opt}$  at 19.5 °C, 20.4 °C and 20 °C for L<sub>2</sub>, L<sub>3</sub> and L<sub>4</sub> respectively. Similarly, appearance of L<sub>2</sub>, L<sub>3</sub> and L<sub>4</sub> of cultivar Khumal-4 had  $T_{opt}$  at 20 °C, 19.8 °C and 19.8 °C respectively. There were not similar response of distinct  $T_{opt}$  for all leaves and  $T_{opt}$  varied among the cultivars. Figure 13 shows the amplitude of temperature responses as

well as temperature sensitivity to leaf appearance decreased with successive leaf.

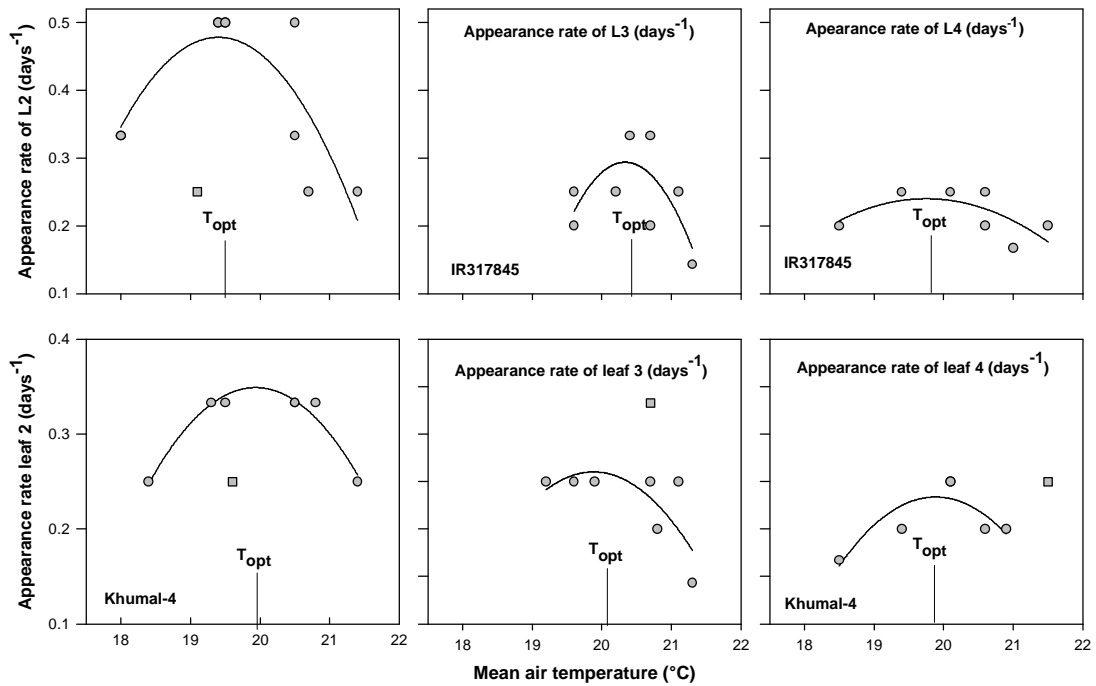


Figure 13. Appearance rates of leaf two, three and four (reciprocal of duration) as a function of mean air temperature for two rice cultivars. Curved line for quadratic regression,  $T_{opt}$ , optimum temperature.

The temperature response patterns changed gradually with successive leaves. There was a curvilinear appearance pattern from  $L_2$  to  $L_4$ , and then the appearance rate was not affected by temperature as shown in figure 14.

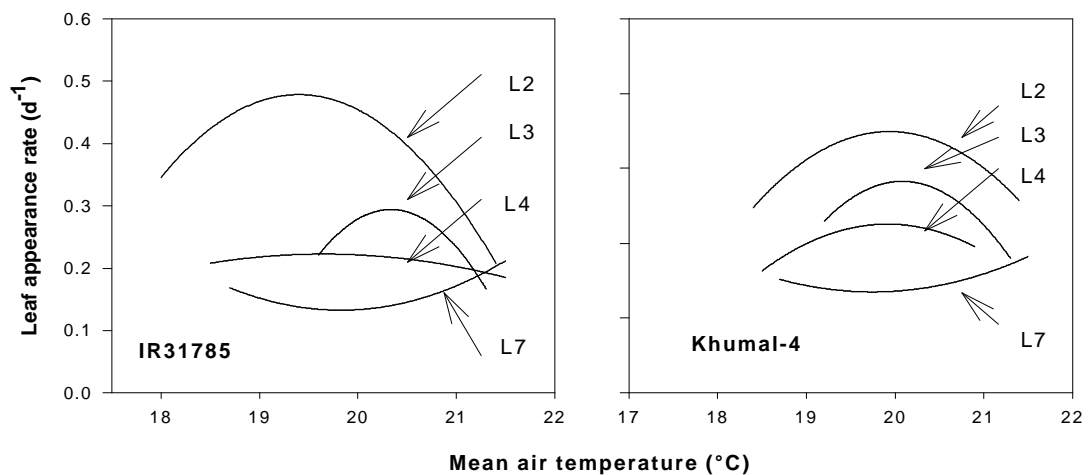


Figure 14. Appearance rate of the 2<sup>nd</sup> leaf (L<sub>1</sub>-L<sub>2</sub>), 3<sup>rd</sup>, 4<sup>th</sup>, and 7<sup>th</sup> leaf of IR31785 and Khumal-4 rice cultivars, as related to air temperature.

## 5.4 Leaf Development and Senescence

### 5.4.1 Leaf development stages at different planting dates

During the experiment, for all successive leaves on the main culm dates were recorded for a particular leaf appearance, 25 % extension, 75 % extension, full extension, onset of senescence, 25% senescence, 75% senescence and death. Based on the available dates, leaf initiation dates were estimated. Individual leaf development was described as senescence (-1 to 1, initiation to death) and physiological activity (source-sink function of the leaf). The different stages of a leaf were defined as initiation (-1), appearance (-0.5), onset of source (-0.25), full extension (0), onset of senescence (0.5), end of source (0.62), 50% senescence (.75) and death (1) as shown in figure 15.

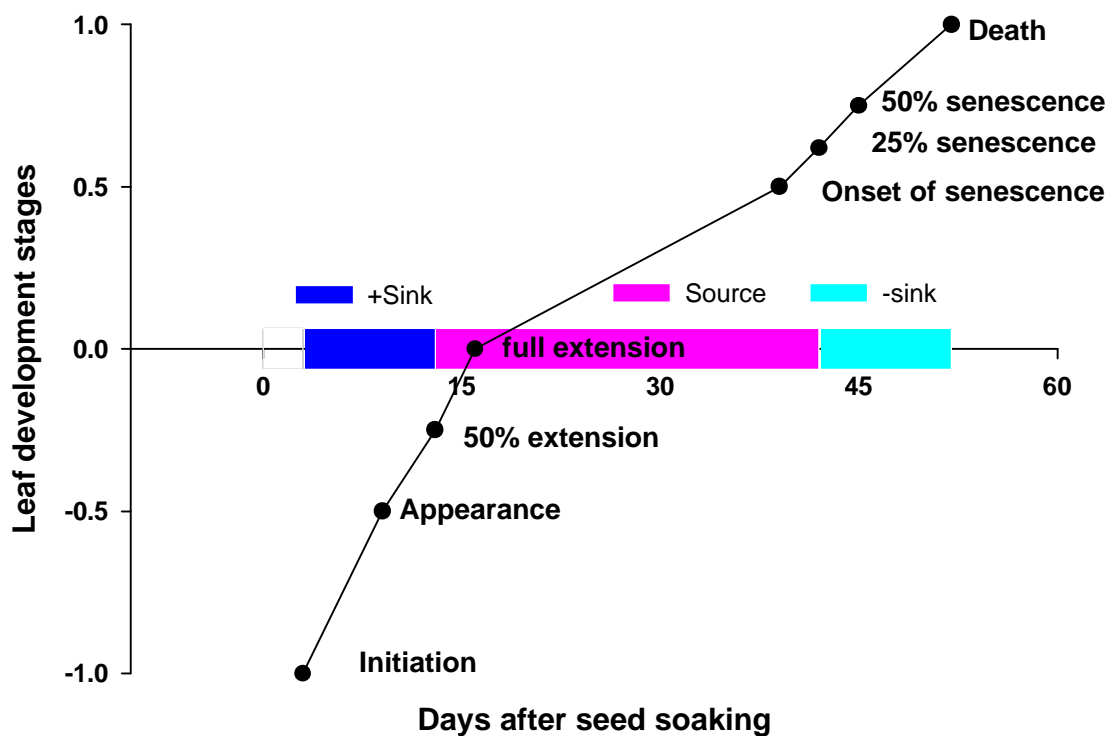


Figure 15. Development stages of leaf 1 on the main culm of cultivar IR31785. Colored boxes show the source and sink phases of the leaf.

Figure 16 shows the development stages of different leaves of rice cultivar IR 31785 at six different planting dates. The figure shows that the active

physiological stage, full extension to the onset of senescence, was increased with later leaves. Early leaves ( $L_1$  to  $L_5$ ) appeared, fully developed and died faster than the later leaves. The active physiological period of leaf 3 at first planting was 28 days which was increased to 37 days at third planting and decreased to 29 days at fifth and sixth planting. Whereas the active physiological period of leaf 9 was 45, 48, 86 and 98 days at first, third, fifth and sixth planting respectively. Hence, the source period was increased with later planting dates. When comparing with a local cultivar Sabitri (Figure 17) similar trend was found for leaf 3 and 9.

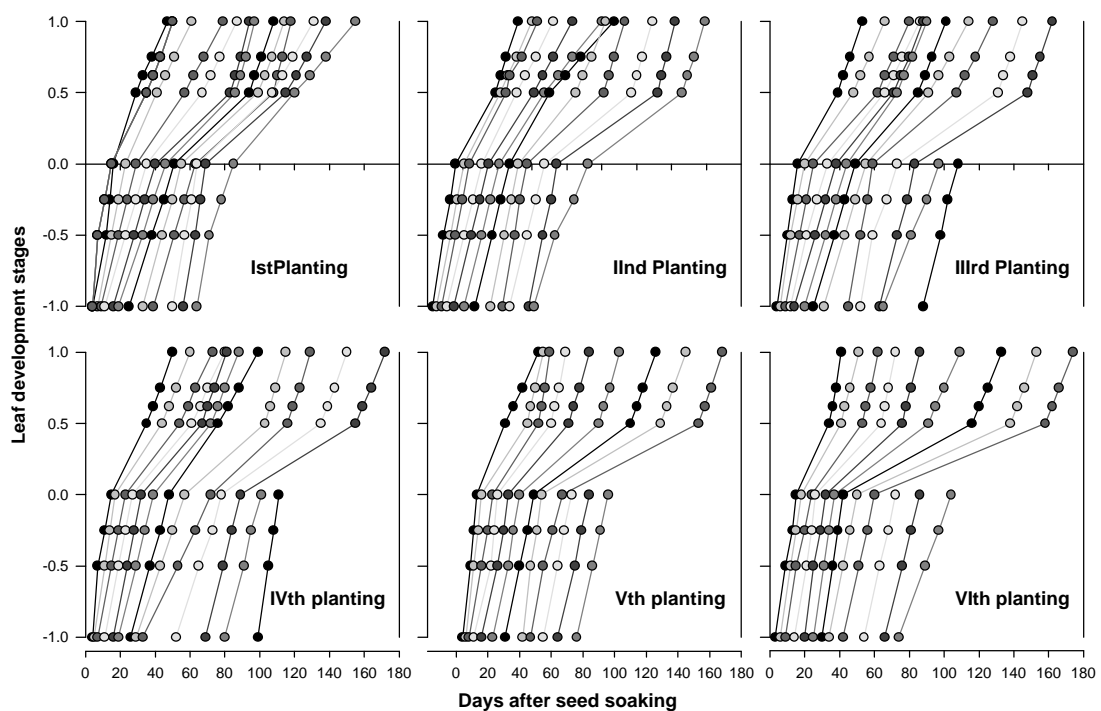


Figure 16. Development stages of different leaves of rice cultivar IR31785 at different planting dates.

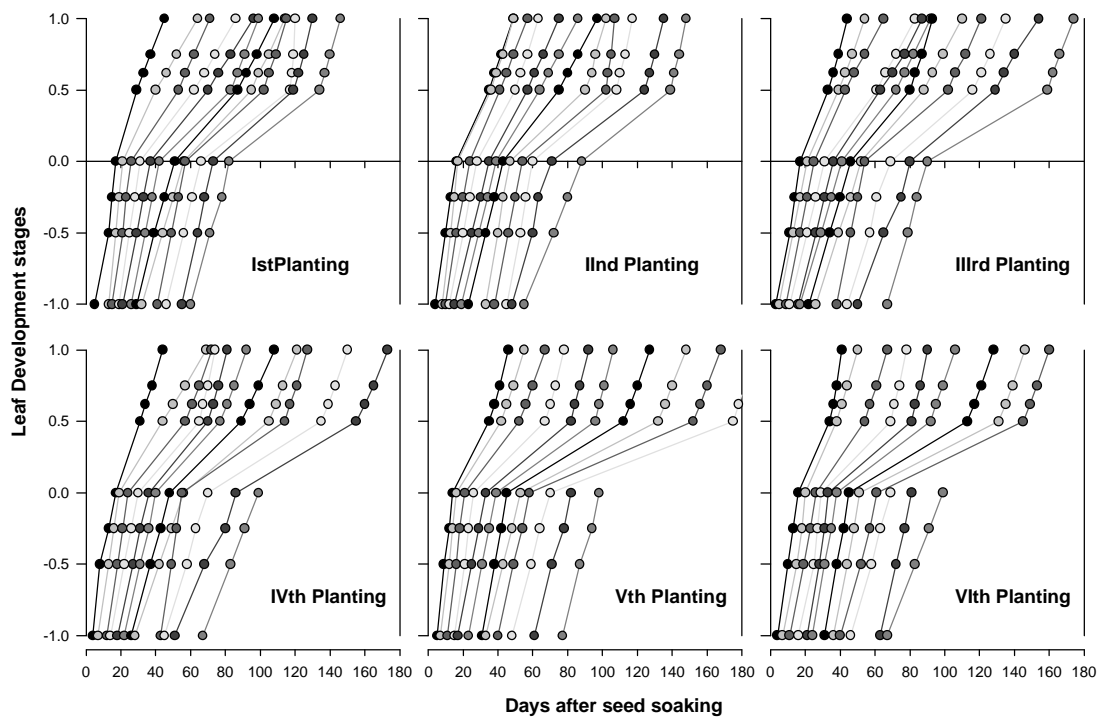


Figure 17. Development stages of different leaves of rice cultivar Sabitri at different planting dates. Figure 18 shows that there were differences in development stages of early leaves among the cultivars tested. For example cultivar Khumal-4 appeared, developed and died earlier than the other cultivars.

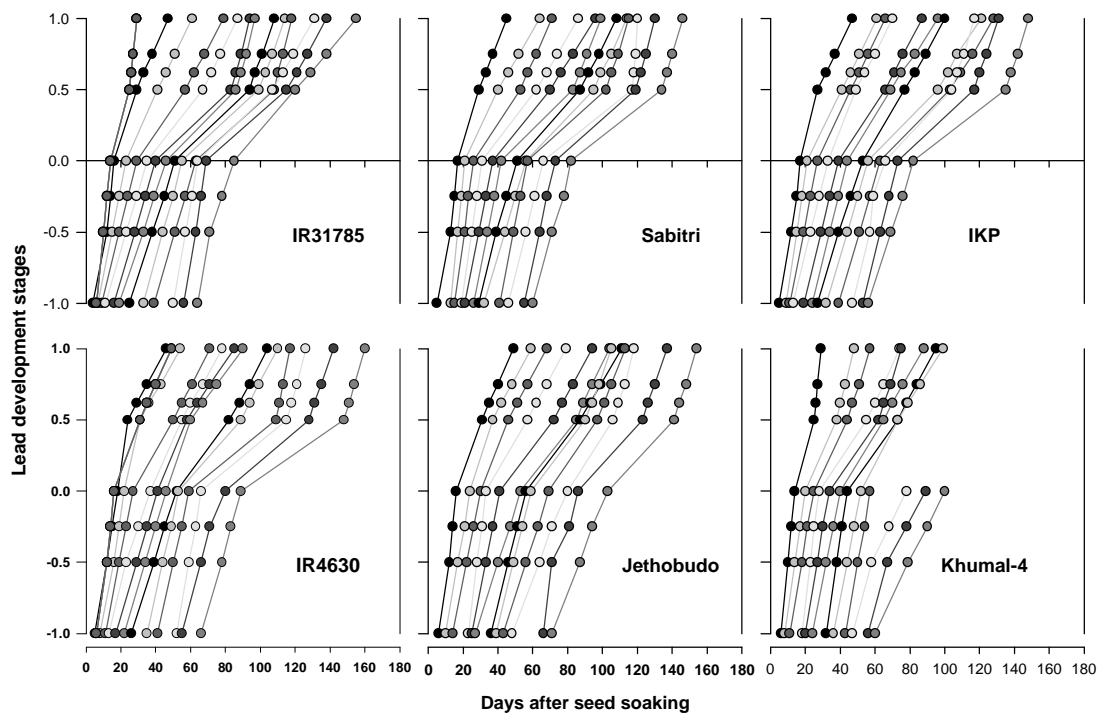


Figure 18. Development stages of different leaves of six different rice cultivars at 1st planting date.

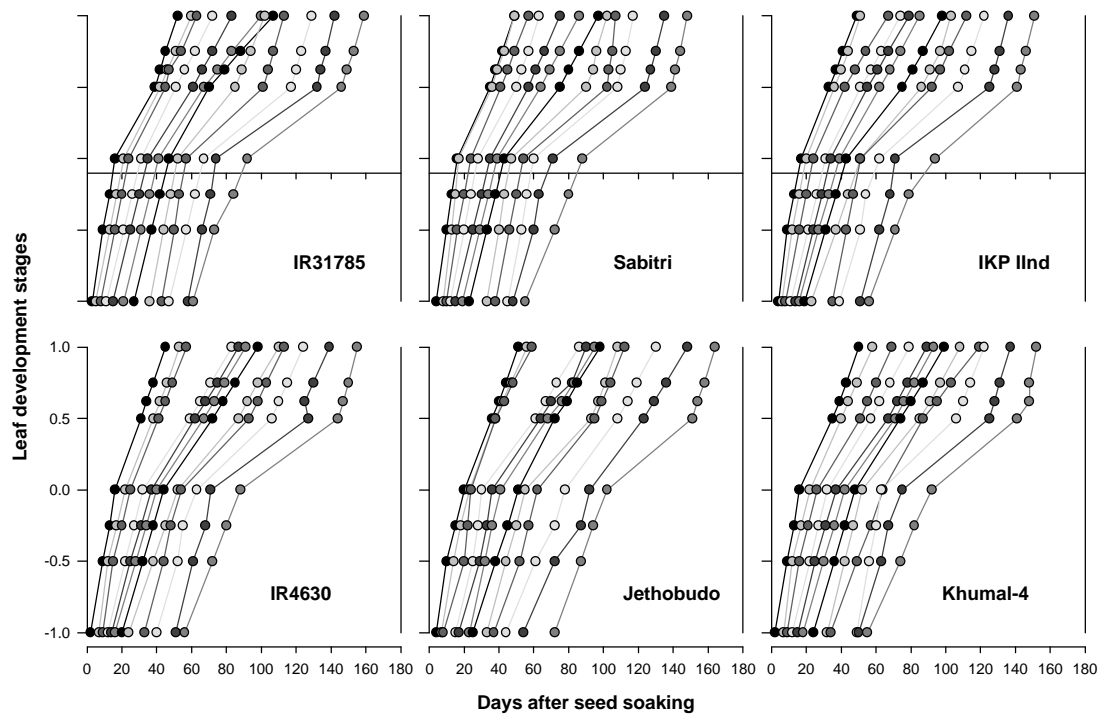


Figure 19. Development stages of different leaves of six different rice cultivars at 2nd planting date. However, from second planting the differences in development stages were not significant.

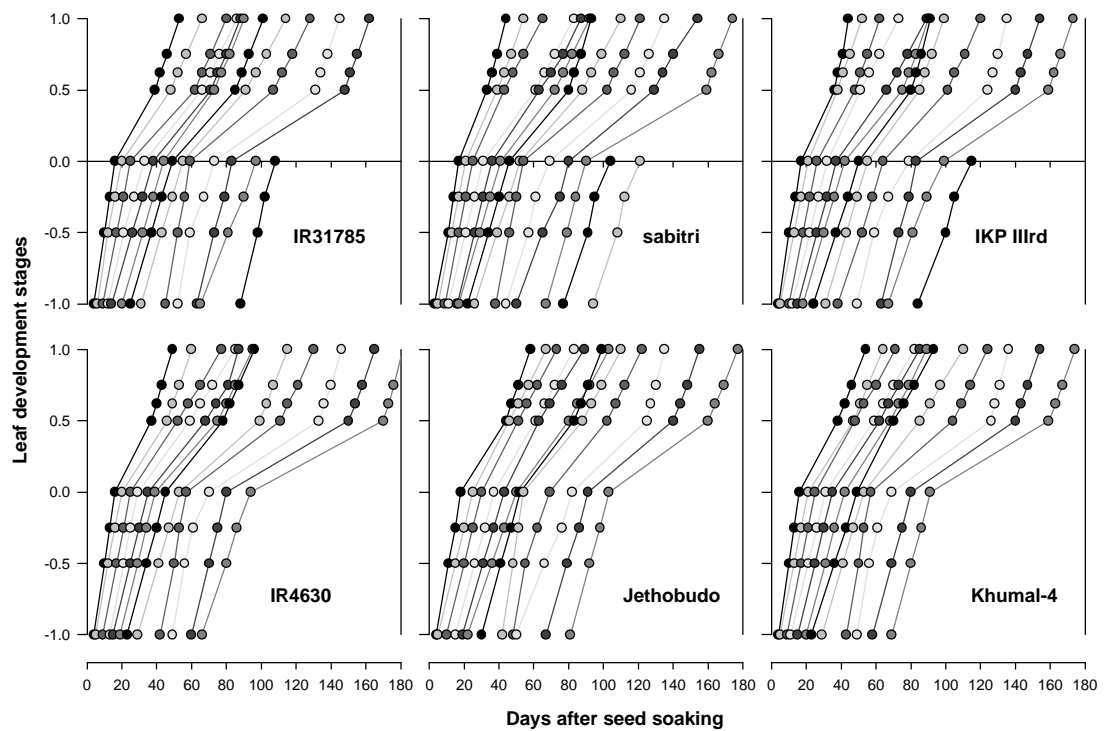


Figure 20. Development stages of different leaves of six different rice cultivars at 3rd planting date.

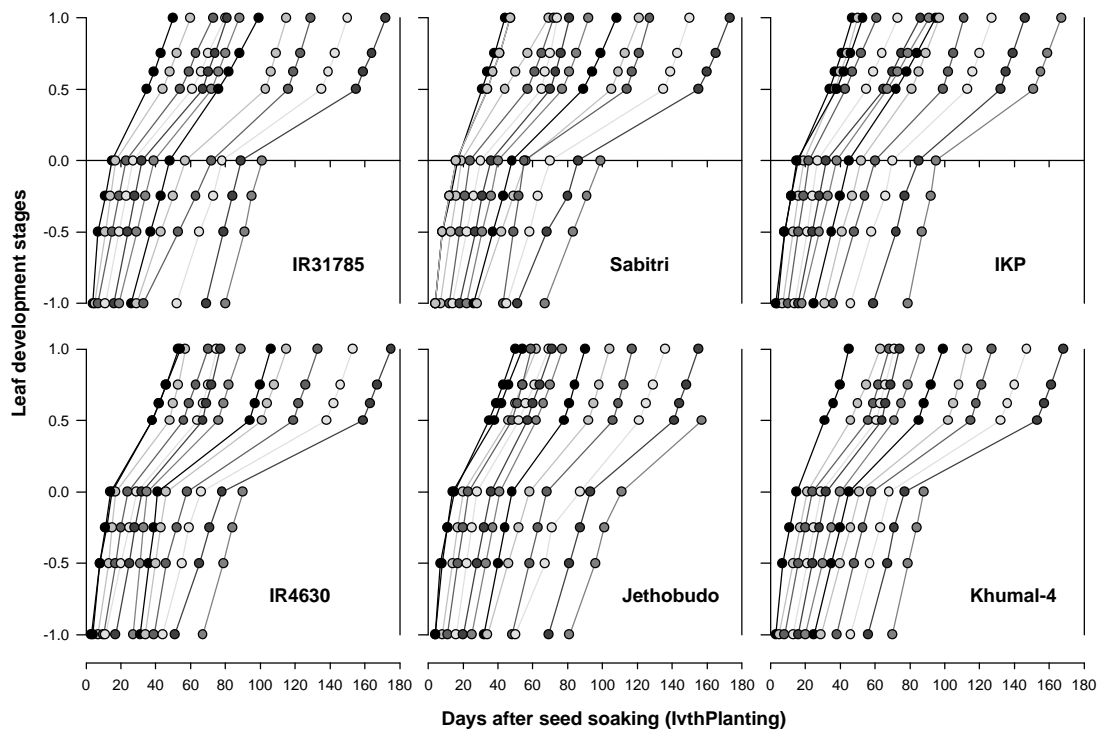


Figure 21. Development stages of different leaves of six different rice cultivars at 4th planting date.

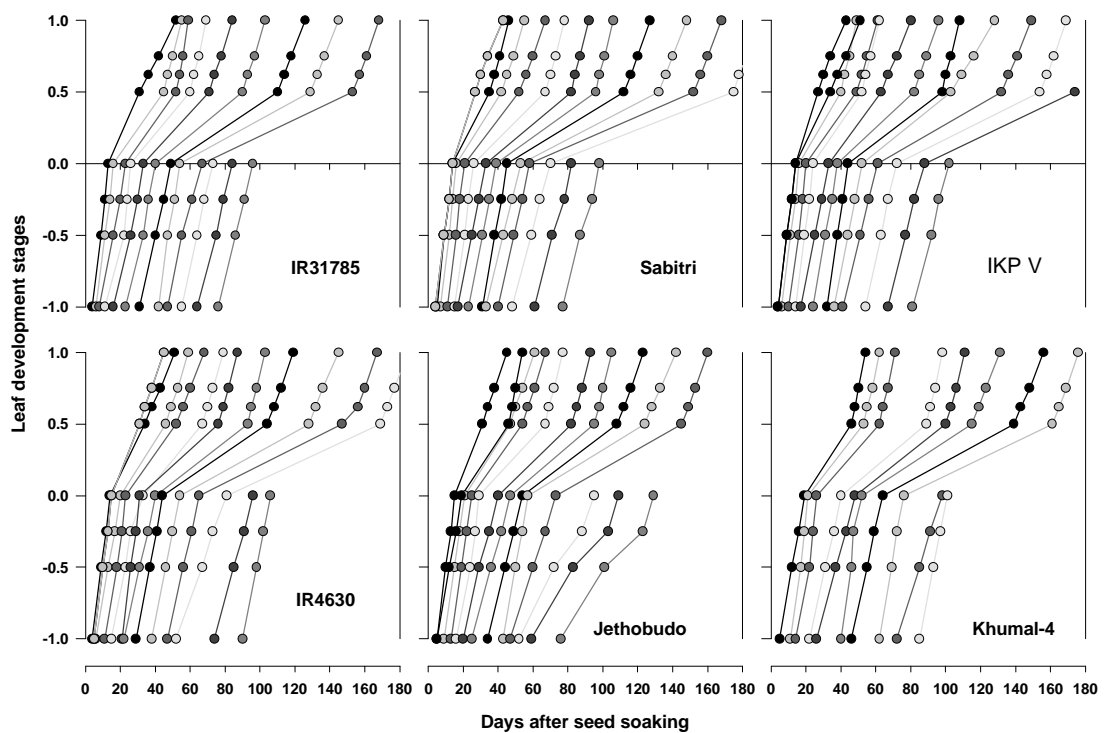


Figure 22. Development stages of different leaves of six different rice cultivars at 5th planting date.

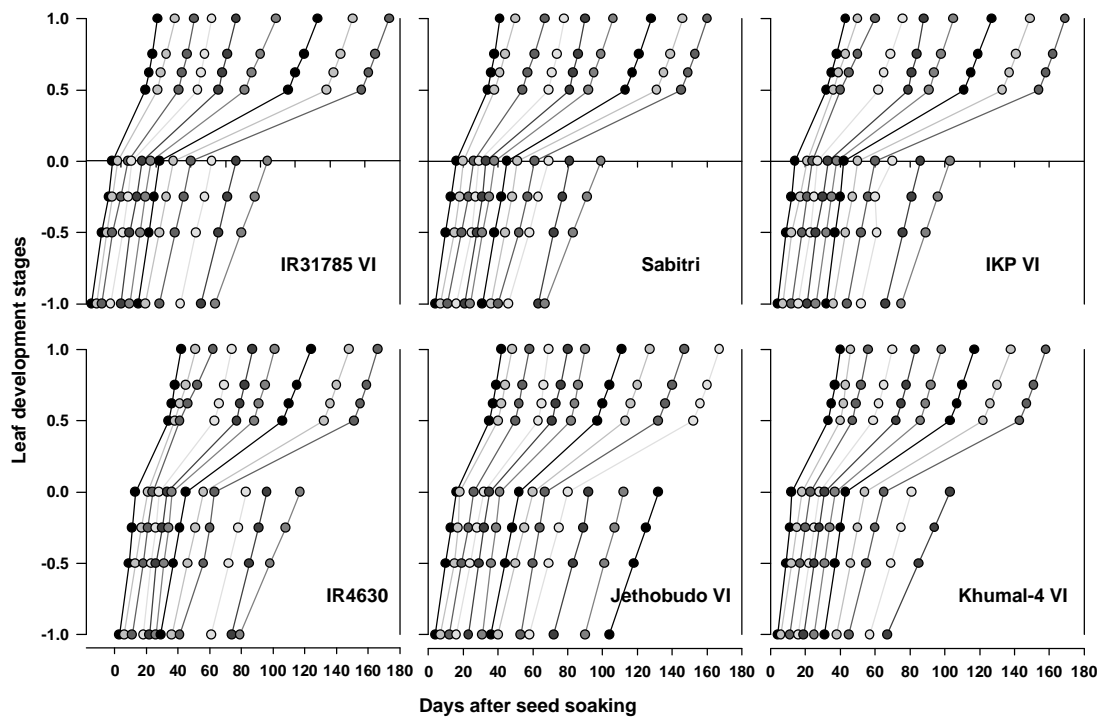


Figure 23. Development stages of different leaves of six different rice cultivars at 6th planting date.

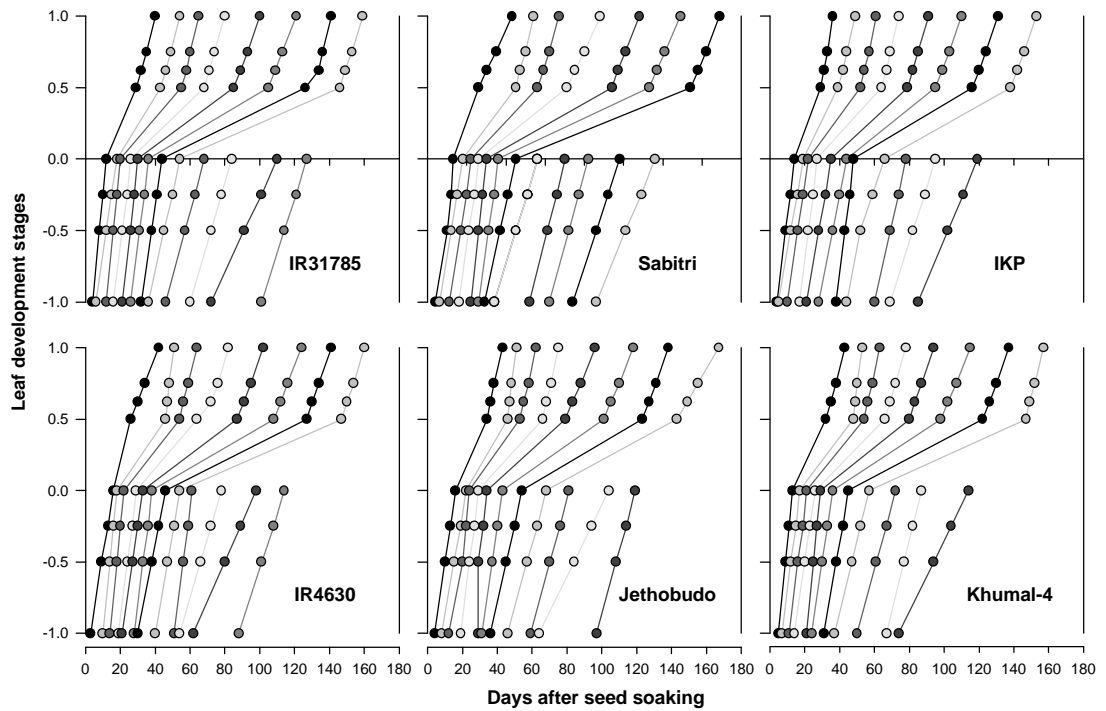


Figure 24. Development stages of different leaves of six different rice cultivars at 7th planting date.

### 5.4.2 Canopy senescence level across the age of rice plant

The development stages of a leaf were defined as initiation, appearance, full extension, onset of senescence and death. From the available data, daily individual leaf development stages were calculated and existing leaves on main culm at leaf appearance were added to calculate canopy senescence level. Figure 25 shows the canopy senescence level of cultivar IR31785 and IR4630 at three different planting dates. The canopy senescence level, excluding dead leaves, 50 days after sowing was on an average of 1.5 for both genotypes at different planting dates (Figure 25). The same figure showed that higher canopy senescence level values were associated with earlier planting dates. At sixth planting date, both cultivars showed leveled canopy senescence level 50 days after seed soaking.

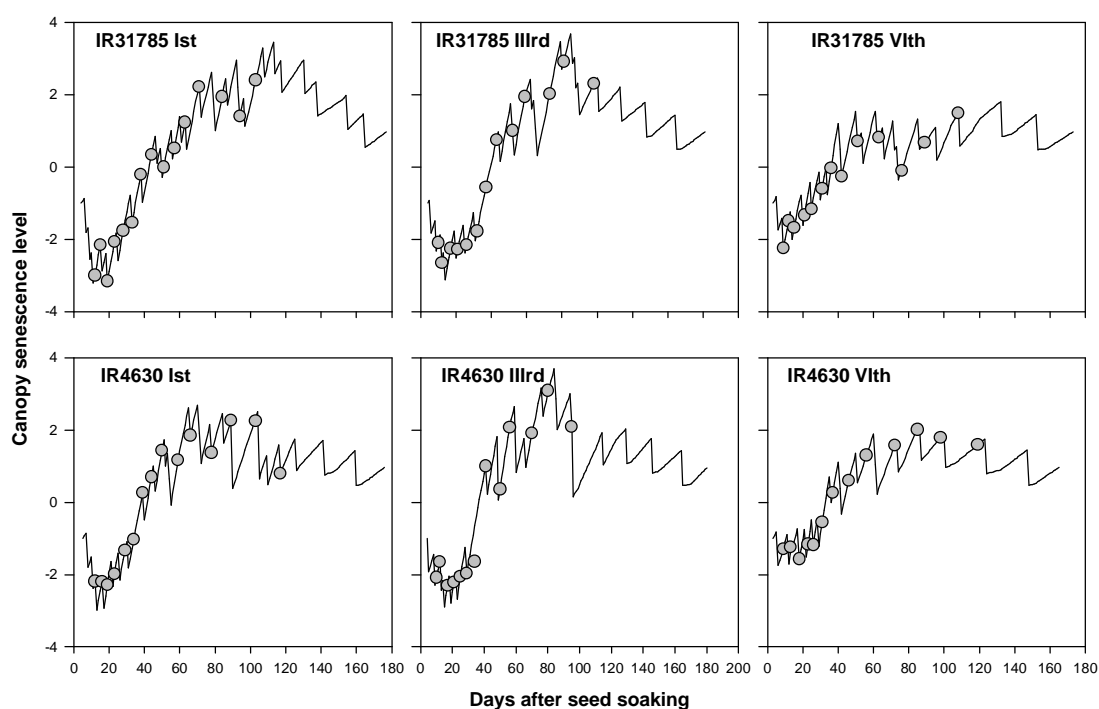


Figure 25. Relationship between canopy senescence level and rice plant age at leaf initiation.

### 5.5 Physiological phases of different leaves

The duration from initiation to the death of a particular leaf was divided into different stages, to compare the physiological period of a leaf, and defined as **sink +** (initiation to onset of source), **source** (onset of source to end of

source) and **sink-** (end of source to death). The percentage period share of these stages were calculated and presented in figure 26.

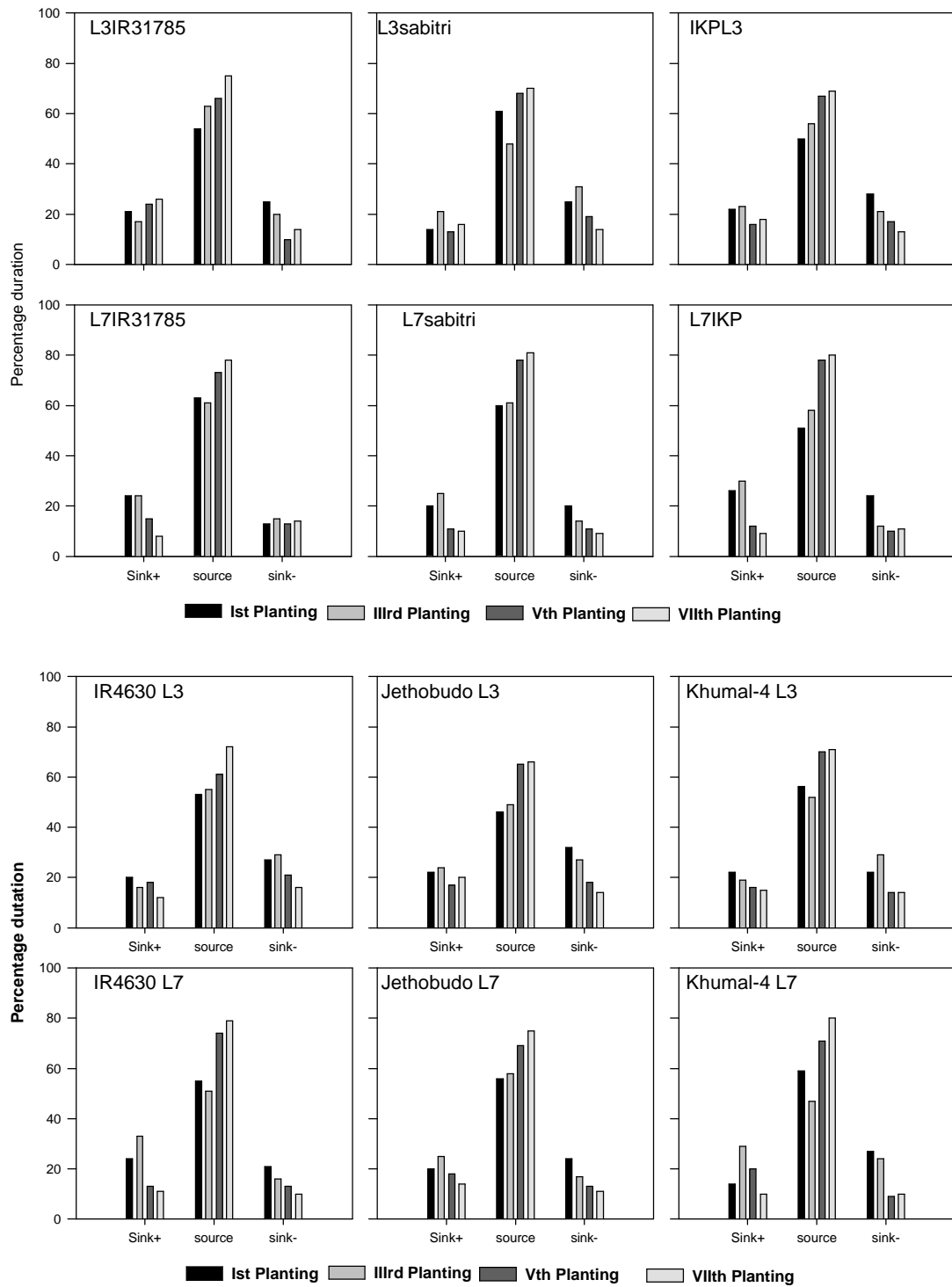


Figure 26. Percentage share of different physiological phases (sink+, source and sink-) relative to the total leaf duration of six rice varieties at four different planting dates.

Figure 26 shows the percentage duration of physiological phases of a leaf varied among planting dates. Figure 26 shows that  $L_3$  and  $L_7$  had increased source duration at later planting dates whereas the sink+ and sink – showed different response. Cultivar Khumal-4 showed increased source duration at first planting for both  $L_3$  and  $L_7$ . The same figure showed that sink- was decreased at later planting dates in most of the cultivars. Sink+ showed mixed response with different planting dates among tested cultivars. Hence, the duration of the source phase of a leaf, which is the most important period for the plant, increased with later planting dates.

## 6 Discussion

Rice-wheat crop rotation is Nepal's most important crop production system. Farmers are practicing this system since a long time. Hence, the fertility status of this system is poor and yields of the crops are either stagnant or decreasing (Maske *et al.*, 2000 and Regmi, 2002). This system is characterized by a more or less pronounced transition period between the dry and wet season (Figure 27). Management of this transition period, by growing transition season crops, leads to nutrient conservation, increased nutrient use efficiency and increased sustainability of the system (Pande and Becker, 2003). In Nepal, farmers adopt different cropping calendars in the Terai and the midhills due to the different climatic environments. In the Terai region, this transition period is comparatively longer than in the midhills, hence, farmers can grow transition season crops during this period without changing the planting or sowing times of dry and wet season crops. In the midhills, this transition period is not sufficiently long to grow transition season crops.



Figure 27. Diagram showing rice-wheat crop rotation and possible changes in cropping calendar.

Growing transition season crops and/or the replacement of the dry season wheat by a crop, like potato, can result in a substantial deviation from the recommended planting dates of rice (Figure 27). Since, rice phenology depends on the photothermal environment; changes in planting date may

influence two major phenological aspects: (1.) the vegetative development and (2.) the reproductive development.

The research site located at the midhill region of Nepal. The climate of the site is characterized by cool dry winters and warm humid summers. Mean air temperature increased from January up to April and remained more or less stable until September. After September the mean air temperature again started to decrease. The recommended transplanting time of rice in the midhills of Nepal starts from 15<sup>th</sup> of June. Here, shift in transplanting time earlier than this date helps to utilize the nutrient available after the first rain. At the same time, shift in transplanting time later than the recommended date helps to grow transition season crop. Thirty two rice cultivars were sown at eight different planting dates with 15 days interval, starting from 24<sup>th</sup> of April as first seed soaking date. Large numbers of rice genotypes were included to test their responses to the changed climatic condition. For this study, 6 cultivars, three from Nepal and three from international germplasm collection, were considered to evaluate their crop establishment responses to variable climate of high altitude.

Leaves are the principle organs of a rice plant. They are the source of carbohydrate for the production of dry matter and yield. Hence, it is important to know the appearance rate and pattern of different leaves, duration of leaves, development stages of leaves and physiological phases of different leaves. Change in planting time may change the vegetative development of rice plant. Due to the length of the rice crop cycle and different planting dates (eight) included in the experiment, the present research work considered only the early establishment of rice crop i.e., vegetative development in relation to different planting dates. Here, this study tried to answer the following questions: (1) what is the effect of change in planting dates on leaf appearance and duration? (2) What is the appearance rate of different leaves under varying temperature? (3) What is the effect of planting dates on leaf development and senescence on different rice cultivars? and (4) Does physiological potential of individual leaf changes at different planting dates?

In this section, the result of the present study will be discussed in view of findings of other researchers related to this experiment.

### 6.1 Effect of planting dates on different leaves

One of the objectives of this study was to assess the effect of different planting dates on leaf appearance and duration of leaf. Hence, dates were recorded for particular leaf appearance, when the leaf tip was visible and death in the field. The duration from seed soaking to the emergence of the first leaf ranged from 7 to 13 days, corresponding to the appearance rates between 0.14 and 0.08 day<sup>-1</sup>. Sie et al., (1998b) also reported similar ranges of appearance rate for leaf 1. The appearance of first leaf was positively and linearly correlated with mean air temperature from seed sowing to the appearance of the first leaf. They reported development rates for the first leaf between 0.25 and 0.08 days<sup>-1</sup>, where the development rate was higher than the development rate of first leaf of present study. They explained higher rate of development due to higher temperature (average water temperature ranged from 16 to 35 °C) experienced by the leaf, which was not possible in present study due to the lower temperature range (mean air temperature ranged from 15.5 to 22.5 °C).

Jaffuel and Dauzat (2004) reported that the number of leaves on the main stem increased linearly over time for 45-50 days after sowing with 10-11 leaves. I found a similar linear relation for number of leaves on main culm over time but the number of leaves was 7-8 (figure 9). Jaffuel and Dauzat (2004) did their experiment in glasshouse with constant day and night temperature of 25 °C and 20 °C respectively whereas this research was done under field conditions. At the same time number of leaves on a culm at any time depends mainly on the resources available, i.e., light, nutrients and space, planting density and fertilizer.

I found a change in leaf appearance pattern at different planting dates (Figure 10). Leaf appearance rates were higher for the early planting dates and leaf durations were short, whereas in the later planting dates, particularly for the later leaves leaf appearance rates and leaf duration increased (Figure 10)

with a clear shift towards a longer productive source period (Figure 26). Sie et al., (1998a) also reported changes in leaf appearance pattern at different planting dates. Sie et al., (1998a) reported early leaf generations (e.g., L<sub>1</sub>-L<sub>4</sub>) appeared faster than the later leaves. I found similar pattern for leaf generations up to L<sub>7</sub> (Figure 10), and later leaves appeared slowly. Sie et al., (1998a) represented a climatic environment of semi-arid conditions of irrigated rice in Sahel and this study represents the climatic environment of midhills of Nepal. Hence, it is clear that the early leaf generation responds differently in different climatic conditions. Yoshida (1981) reported an average duration between successive leaf appearances of 4 to 5 days before panicle initiation and 7-8 days afterwards. In our experiment, we found an average duration of 4.3 days for L<sub>1</sub> to L<sub>4</sub> and of 8.8 days for L<sub>7</sub> to L<sub>12</sub>. Rice cultivar Khumal-4 showed earlier and faster rate of leaf appearance at the early planting dates. Hence, there is chance to grow Khumal-4 in the early planting dates.

## 6.2 Effect of temperature on different leaves

Rice produce 10-18 leaves during its life cycle and this number vary across the genotypes. Appearance of individual leaf is influenced by many factors. Among these factors temperature is the main factor controlling the leaf appearance. In this study, we found differential responses of leaf appearance rate of individual leaf with temperature. Whereas, Ritchie et al., in Birch et al., (1998) reported that temperature controls the rate of leaf appearance. Sie et al., (1998b) reported that the development rate of first leaf was linearly correlated with water temperature across and within all lines. In the present research, we found only first leaf showed such relation with mean air temperature across six rice cultivars (Figure 12).

Sie et al., (1998a) showed that the appearance rate of the first to the fourth leaves followed a parabolic temperature response with a distinct  $T_{opt}$ . In this experiment, a parabolic temperature response of rice cultivars IR 31785 and Khumal-4 with the appearance rate of L<sub>2</sub>, L<sub>3</sub> and L<sub>4</sub> was found. Furthermore, each leaf showed a distinct  $T_{opt}$  with the appearance rate (figure 13). Sie et al.

(1998a) reported higher  $T_{opt}$  than the present finding in the IR series rice cultivars. They reported a range of  $T_{opt}$  from 22 to 26 °C, but we found  $T_{opt}$  from 19.5 to 20.5 °C. Sie *et al.*, (1998a) reported no significant temperature dependency on leaf appearance rates for leaf position more than 5. They reported that between the appearance of  $L_1$  and  $L_6$ , temperature response patterns changed gradually in the test cultivars from a steep and linear response, to a curvilinear pattern demonstrated for LR1-4, and then to a constant appearance rate that was unaffected by temperature. I found the same pattern of response for rice cultivar IR31785 and Khumal-4 for the appearance of  $L_2$ ,  $L_3$ ,  $L_4$ , and  $L_7$  (Figure 14). Here, the concept that temperature controls leaf appearance does not explain the appearance of later leaf generation. Das (2004) reported a salinity induced leaf appearance in rice at same thermal condition in greenhouse experiment Hence, there may be some factors, other than temperature, that affect initiation of later leaves. Birch *et al.*, (1998) reported that phyllochron (time interval for leaf tip appearance) in maize is related to light intensity and irradiance. Das (2004) reported a canopy senescence level as an indicator for a rice plant to initiate new leaves.

### 6.3 Leaf development and senescence

Leaf development stages were defined as initiation (-1), appearance (-0.50), 25% extension (-0.25), full extension (0.00), onset of senescence (0.50), 25% senescence (0.62), 50% senescence (0.75) and death (1) as shown in figure 15. We found that the leaf development stages were different at different planting dates. The duration from appearance to the onset of senescence is increasing at successive planting dates (Figure 16).

There are arguments about leaf appearance in rice crop. Some researcher reported leaf appearance is controlled by temperature and others reported other factors control leaf appearance. We tried to relate leaf initiation with the canopy senescence level of the rice plant. Birch *et al.*, (1998) reported that the phyllochron depends on the adequacy of current photosynthesis (source) to meet the demands of the plant for growth (sink). Hence, when the overall

canopy senescence level of a tiller exceeds a certain value, the plant initiates a new leaf for the maintenance of a functional canopy to supply the required carbohydrates to the plant. In our study, we tried to calculate the value of canopy senescence level and found that there were similarities among the cultivars tested at different planting dates (Figure 26). Das (2004) reported a canopy senescence level of 2 for the initiation of new leaf on rice tiller. As this value is based on the leaf initiation, which is an estimated value, hence we tried to find the relation with leaf appearance. We found the appearance of new leaf when the spike of canopy senescence level was in its maximum; however, this relation was not uniform in all cultivars.

#### 6.4 Physiological phases of different leaves

The fourth objective of the study was to examine the physiological phases of different leaves at different planting dates. From the initiation to the death of a leaf, it has differential physiological potential. Hence, the leaf development stages were divided into three phases, based on source-sink period, namely; 1.) Sink+ (leaf initiation to 25% extension) 2.) Source (25% extension to 25% onset of senescence) and 3.) Sink- (25% onset of senescence to death). In our experiment we tried to relate these physiological phases across different planting dates. It was found that the source duration increased at later planting dates for all tested cultivars (Figure 26), whereas the sink- decreased with later planting dates with some variation. The sink+ duration did not show clear change in percentage duration. Dahal (2004) reported a decreased duration of source period of early and late leaf numbers with salinity treatments in rice plant. Here, salinity reduced the potential source duration in rice leaf. So, what is influencing the increased source duration of leaf in rice? In this experiment, the temperature at the later part of the experiment was decreased; hence the temperature may had influenced the source duration in rice.

## 7 Conclusion

This study was conducted to examine the leaf appearance pattern and leaf duration at different planting dates; to assess the appearance rate of different leaves in relation to temperature; and to explain the effect of different planting dates on leaf development stages.

Planting date influenced leaf appearance rate decreasing the total number of leaves on the main culm at later planting dates. Early leaf generations (L<sub>1</sub>-L<sub>5</sub>) appeared faster than later leaves, irrespective of planting dates; with leaves of Khumal-4 cultivar appearing faster than those of the other cultivars at all planting dates. Early leaves showed temperature dependency; with increasing mean air temperature reducing the number of days required for the first leaf to appear for all cultivars tested. However, the appearance rates of leaf L<sub>2</sub>-L<sub>4</sub> showed distinct optimum temperature, below and above which there was pronounced decrease in leaf appearance rate. The temperature response patterns reduced gradually with successive leaves and from L<sub>5</sub> onwards the appearance rate was not dependent on temperature. Development stages of leaves were affected differentially by planting dates. With the exception of Khumal-4, which had an increased duration of active physiological phase at early planting dates, the active physiological phases of all cultivars increased only at the later planting dates. There may be factors other than temperature influencing the appearance and development of later leaves.

As this study covers only the vegetative development of rice in relation to change in planting time, it would be premature to conclude a recommendation for farmers. However, considering the early crop establishment parameter, this research found that rice cultivar Khamal-4 can be grown under early planting situation due to its high leaf appearance rate, low optimum temperature and long physiological phases during the early planting dates. Recommendation for farmers should be based on the reproductive performance of the rice genotypes.

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## Appendix

Table 2. Weekly maximum, mean and minimum temperature (°C) observed during the research period at the experimental site (18<sup>th</sup> April to 31<sup>st</sup> December 2004)

Number of weeks	Maximum temperature (°C)	Mean temperature (°C)	Minimum temperature (°C)
1	24.6	17.3	9.9
2	25.3	18.1	11.3
3	25.5	18.4	11.2
4	26.2	19.2	12.2
5	26.7	19.4	12.1
6	26.3	19.3	12.0
7	24.5	19.1	14.2
8	25.2	19.8	14.4
9	25.3	19.3	13.3
10	24.7	20.3	15.9
11	25.2	20.7	16.2
12	24.3	20.7	17.2
13	22.9	20.0	17.0
14	23.2	20.6	18.0
15	24.5	20.8	17.1
16	24.4	20.9	17.4
17	25.1	21.5	17.9
18	25.3	21.4	17.6
19	24.8	21.0	17.1
20	24.3	21.0	17.6
21	23.8	20.5	17.2
22	22.1	18.9	15.7
23	23.2	19.5	15.9
24	21.5	17.8	14.1
25	21.4	18.0	14.6
26	21.1	17.0	12.9
27	21.1	16.0	11.0
28	19.7	14.8	10.0
29	19.2	13.9	8.6
30	18.7	13.5	8.3
31	17.2	12.9	8.5
32	16.5	12.1	7.8
33	16.9	11.5	6.1
34	18.3	12.5	6.8
35	16.4	12.4	8.4
36	15.0	11.0	7.0
37	12.9	8.8	4.6

## Curriculum Vitae

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### **Education and Qualification**

#### **University of Bonn, Agricultural Science and Resource Management in Tropics and Subtropics (ARTS)**

*Master of Science in Agriculture*

#### **Tribhuvan University Institute of Humanities and Social Sciences Tri-Chandra Campus, Nepal**

*Master's Degree in Sociology*

#### **Tribhuvan University Institute of Agriculture and Animal Sciences (IAAS), Rampur, Campus Nepal**

*Bachelor's Degree in Agriculture (Four Years)*

#### **Tribhuvan University, Institute of Management (Privet)**

*Bachelor's Degree*

#### **Tribhuvan University, Institute of Agriculture and Animal Sciences (IAAS), Paklihawa campus, Nepal**

*Proficiency Certificate in Agriculture*

#### **Shree Jhuwani Secondary School, Chitwan, Nepal**

*School Leaving Certificate (SLC)*

### **Experiences**

#### **Jan 1999- Present**

##### **Department of Agriculture of His Majesty's Government of Nepal**

*Crop Development Officer*

Responsible for research and extension of field crops suitable for different agro-ecological zones of Nepal. Planning and monitoring of extension activities.

#### **May 1998- Dec. 1998**

##### **Nepal Agriculture Research Council, Nepal**

*Technical Officer (Agronomist)*

Responsibilities were: Conducting research activities, data recording and analysis.

#### **Jan. 1998- April 1998**

##### **East Rapti Irrigation Project Study Group Rampur, Nepal**

*Research Assistant*

Responsibilities were: Data collection, data entry and analysis.

**Publications**

Khanal, R.R., Asch, F., Becker, M. 2004. Phenological responses of rice cultivars under varying thermal environments in a high altitude cropping system. Deutscher Tropentag 2004, 5-7. October 2004, Berlin. Poster

Khanal, R.R., Bhandari, P., Paudel S. 1996. Utilization pattern and Introduction of Elephant Grasses on Farmers field in Jhuwani, Eastern Chitwan. IAAS Research Reports. Institute of Agriculture and Animal Sciences (IAAS) Rampur Chitwan Nepal.

**Additional Information****Language Proficiencies:**

Nepali: Mother tongue

English: Fluent written and spoken

Hindi: Fluent spoken

German: Poor

**Computing:**

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**Private Activities**

- Wild life and Bird watching
- Trekking
- Cycling
- Meditation