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au sein de l'UPR AIVA (Adaptation Agro-écologique et Innovation Variétale)
TA A-104 / 01. Avenue Agropolis. 34398 Montpellier Cedex 5 – France

et à l'IRRI,
College, Los Baños, Laguna 4031 – Philippines

Sur le thème :

Établissement du cadre de développement de l'allongement des organes d'une plante de riz

Pour l'obtention du :

DIPLÔME D'AGRONOMIE GÉNÉRALE

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INTRODUCTION

Internship conditions

This report concerns the internship I conducted during the second part of my 'gap year', in the first semester of 2010. I was registered at Cirad but started this internship in the Philippines, in the International Rice Research Institute (IRRI), located in Los Baños, a small town in the province of Laguna, in Luzon, the largest island of the country where Manila is located. The experiments took place in the 252-hectare experimental farm of IRRI (Picture 1) from January until May, which corresponds to the dry season. I stayed during this whole period there in order to characterize the growth of the rice plants in the field from sowing to maturity and collect and encode data. Then I went back to France for two months to analyze these data at Cirad in Montpellier.

Picture 1: View of the experimental farm of IRRI

CIRAD

CIRAD is the **Center for International Cooperation in Agronomic Research for Development** (Centre de coopération Internationale en Recherche Agronomique pour le Développement). It is a French research center, created in 1984, whose part of the mandate is to work with developing countries to address international agricultural and development issues. In response to the economic, social and environmental changes resulting from globalization, CIRAD has renewed its scientific strategy, which now focuses on six priority lines of research (Ecological intensification; Biomass energy and societies in the South; Accessible, quality food; Animal health and emerging diseases; Public policy, poverty and inequality; Agriculture, environment, nature and societies), associated with a new geographical partnership strategy aiming at expanding and consolidating its international cooperation structure.

CIRAD has a staff of 1800, including 800 researchers. It has an operating budget of 203 M€, with two thirds provided by the French government. It comprises three scientific departments (Biological Systems, Performance of Tropical Production and Processing Systems, Environments and Societies) and 46 research units. It also has 12 regional offices in metropolitan France, the French overseas regions and other countries.

I was part of the **Biological Systems** department (BIOS) which works on a genome, cell, organism and population level, centering its research on the **interactions between genotypes and with their environment**, drawing upon concepts and tools from the fields of biochemistry, genomics, molecular physiology, statistics, genetics, epidemiology, ecophysiology, modeling and breeding. This involves characterizing the genetic diversity of species, of related pests and diseases and of microorganisms of interest in agriculture, identifying and using the genes that control characters of interest in agriculture, characterizing interactions between plants/crops and their environment and plants/crops response patterns to given growth conditions, implementing genetic improvement and propagation techniques, and studying biotic interactions, **combining analysis and modeling** so as to gain a clearer understanding of biological systems and to develop tools to improve them.

BIOS is composed of 18 research units, among them the **Agro-ecological Adaptation and Varietal Innovation** Unit (UPR AIVA), where I worked.
The objective of this unit is to identify **traits of interest**, characterize the **phenotypic diversity** and the **genotype x environment x management interaction**, to contribute to the improvement of plant materials and cultural practices with help of new tools in molecular biology, physiology and informatics. Its main scientific topic is **genotype adaptation to environmental constraints**: climatic factors, inter and intra-specific competition and societal and technological change. The unit is involved in various improvement programmes concerning mainly **rice** (Colombia, Brazil, Philippines, Senegal), **sorghum** (Senegal, Mali, Colombia) and **oil palm** (Benin, Indonesia). Its research activities are driven by a systemic approach involving studies of biological mechanisms, the development of innovative methodologies (modelling and biostatistics tools, molecular markers, etc) and the creation or selection of new varieties.

CIRAD has scientific partnerships with most of the international agricultural research centers in the Consultative Group on International Agricultural Research (CGIAR), notably with IRRI. My supervisor, Dr Tanguy Lafarge, CIRAD researcher and senior scientist from the Crop and Environmental Science Division at IRRI, worked in the Philippines from 2002 until March 2010.

IRRI

IRRI is the **International Rice Research Institute** whose mission is to reduce poverty and hunger, improve the health of rice farmers and consumers, and ensure environmental sustainability through collaborative research, partnerships, and the strengthening of national agricultural research and extension system, summed up in its catch phrase: *'Rice Science for a Better World'*. It celebrated this year its 50th anniversary.

For instance, IRRI delivered climate change-ready rice tolerant to drought and submergence and discovered genes to speed up the delivery of salt-tolerant rice varieties. IRRI is notably trying to increase the efficiency of rice photosynthesis by developing a C4 rice plant. This institute helps conserving rice genetic diversity by maintaining more than 109,000 types of rice at the International Rice Genebank and freely shares rice know-how online through the Rice Knowledge Bank.

IRRI has a staff of about 1,300 mostly located at the headquarters in the Philippines but also in offices in 12 Asian countries and two African countries. It is a nonprofit organization that sources funding worldwide from governments, philanthropy, the private sector, and through the CGIAR. In 2009, IRRI's budget was US$ 54 million.

I was hosted by the **Crop and Environmental Science** Division (CESD) who conducts research in crop, soil, water, and environmental sciences to ensure sustainable, resource-use-efficient, high-yielding, and environment-friendly rice ecosystems.

Rice

Rice is the longest, continuously grown cereal crop in the world. There are about 120,000 varieties known to exist. Rice is the common name for the genus Oryza, belonging to the Poaceae family. **Oryza sativa** (Asian rice) is the most cultivated species. It comprises two subspecies: indica and japonica.

Rice is highly adaptable and can be grown in diverse environments: in upland, irrigated, rainfed lowland or flood-prone areas. However, it grows best when submerged in water. Irrigated rice produces about three quarters of all rice harvested whereas it represents only 55% of the total area. This crop can be grown after transplanting or direct seeding.

The growth of the rice plant can be divided into three phases (see annex 1 for length in time of these phases):

- The **vegetative stage** is characterized by active tillering (when shoots, called tillers, arise from the main culm or stem), gradual increase in plant height, and leaf emergence at regular intervals.
- The **reproductive stage** is characterized by culm elongation (which increases plant height), decline in tiller number, emergence of the flag leaf (the last leaf), booting (swelling of the flag leaf sheath during the latter part of the panicle development stage), heading (emergence of the panicle out of the flag leaf sheath), and flowerings (when the anthers of the terminal spikelets protrude and shed pollen).
- The **ripening stage** is characterized by leaf senescence and grain growth.

Temperature, solar radiation, and rainfall influence rice yield by directly affecting the physiological processes involved in grain production, and indirectly through diseases and insects.

It takes about 90 to 200 days for a rice crop to mature. It finally reaches 0,6 to 2 meters tall, and yields from 1 to 9 tons per hectare, depending on variety, growth and climate conditions.

Rice and climate change and variability

Agriculture is one of the sectors that is highly vulnerable to **changes in climate and its variability**. Indeed, **air temperature** and **carbon dioxide concentration** ($[CO_2]$) are two factors that drive plant physiology, i.e. photosynthesis. Then it is important to understand the response of crops to increases and variations in temperature and $[CO_2]$.

Extensive research has been conducted on the effects of **high temperature and temperature variation** on gramineous plants growth. For example, **leaf emergence rate** in barley increases with increasing temperature until an **optimum temperature** and then decreases slowly with further increasing temperature (Tamaki *et al.*, 2002). The effect of high temperatures on spikelet fertility is also well known. For instance, Jagadish (2007) measured that spikelet fertility was reduced by about 7% per °C > 29.6°C in IR64 rice plants.

An **increase in [CO2]** can have an indirect effect on **spikelet sterility**. Indeed, Yoshimoto (2005) conducted experiments through FACE (Free Air CO2 Enrichment) and showed that CO2-induced stomatal closure reduces transpiration, which causes a rise of leaf temperature. Thus, with elevated CO2, the probability of heat-induced spikelet sterility (HISS) of rice increases. He also observed that the increase in panicle temperature due to elevated CO2 was more pronounced at the ripening stage than at flowering, which might affect the quality of rice seeds as well as HISS.

On the other hand, **high [CO2]** can have **positive effects on grain yield**. Liu (2008) conducted experiments on hybrid indica rice (Oryza sativa L.). He concluded that elevated [CO2] had no effect on phenology, but had a significant impact on each of the four yield components (i.e. the panicle per square meter, spikelet

number per panicle, filled spikelet percentage and individual grain weight), resulting in a substantial increase in grain yield (+34%). Such results were also obtained on conventional japonica varieties, but this positive effect of elevated [CO_2] was less pronounced than on hybrid varieties (Lafarge *et al.*, 2010).

Hasegawa's study (2004) demonstrated that **a contrasting effect on blade length to elevated CO2 exists among rice genotypes**. In his experiment, elevated CO2 had a positive influence on blade length in an early variety but a negative influence in medium and late varieties.

The positive response of C3 plants like rice to elevated [CO2] can be seen as a crucial mechanism to **compensate** or even supersede detrimental effects of future climatic conditions. Hence, understanding the **interaction of elevated [CO2] and higher temperature** in view of possible improvements of rice germplasm for adaptation to climate change requires an **integrative modeling approach** where the effects of component traits of interest governing the phenotypic plasticity of yield formation are accounted separately and for contrasted conditions (Lafarge *et al.*, 2010).

Modelling

A model is a description of a more or less complex entity or process. This description can be mechanistic, empirical or hypothetical. It is an abstraction that simplifies reality, disregarding some characteristics considered less meaningful than others. It enables to test hypotheses on the behavior of a system or entity, based the previous formalization of component processes into equations. Modelling is then useful to formalize knowledge on a given system. It is of particular importance in biological domains, because of the complexity of the systems studied, such as plant morphogenesis and its plasticity, according to genotype X environment interactions.

In this perspective, the AIVA research unit of CIRAD created the **model *EcoMeristem*.**

EcoMeristem is a partially **mechanistic plant growth model** that simulates plant (cereals: rice, sorghum and more recently sugarcane) morphogenesis (organ appearance and growth) depending on genotypic parameters (controlling potential morphogenesis, such as organ appearance rate, organ potential size) and environmental conditions (temperature, radiation, water, evaporative demand). In particular, the nutritional status of the plant is daily simulated using a state variable, IC, **index of competition** computed as the overall carbohydrates supply and the cumulated organ demand (depending on the number of growing organ, their growth rate at a given day). It assumes that supply of assimilates regulates demand for assimilates resulting from the production of new organs and conversely, organ production (leaves) feeds back on supply (assimilation) (Luquet *et al.*, 2006). An important originality of the model is to consider carbohydrate storage, meaning that a carbohydrate reserve will be daily updated (filled or mobilized) depending on the supply / demand ratio.

This model formalizes thus in a simple way the functioning of apical meristem, i.e. the GxE (genotype x environment interactions) regulation of organ initiation, pre-sizing and/or abortion (Soulié *et al.*, 2010).

To further deal with the impact of climate change an fluctuations on the performance of the rice crop, *EcoMeristem* is currently improved and coupled with **3D** and microclimate computation tools in the modelling and simulation framework in plant ecophysiology called OpenAlea (Soulié *et al.*, 2010).

Then it can be considered as a **functional-structural plant model (FSPM).** Models with a similar working scale already exist but with different basic concepts: it is the case of the model GREENLAB that simulates resource dependent plasticity of plant (maize, wheat, rice...) architecture thanks to purely mathematical algorithms describing source-sink relationships in the plant (Guo *et al.*). However, this model doesn't rely much on physiology. ADEL (Architectural model of DEvelopment based on L-systems) is another FSPM designed for simulating the 3D architectural development of the aerial part of wheat and maize plants. It is

very precise but doesn't integrate biomass (carbon assimilation and partitioning) information (Fournier *et al.*, 2004).

EcoMeristem can be used to identify which values of model parameters, and thus which 'virtual' genotypes, will be more productive under specific climate conditions. Thus it can be used in **varietal selection or plant ideotype designing**. It can also be used to predict the behaviour of a given genotype in a given environment (once known the parameter values for a given genotype). It can also be used within **a phenotyping approach**: for this purpose, phenotypic data (measured) characterizing a large number of genotypes (collection, population) are given to the model as target values to be simulated in targeted environment; each genotype will thus be characterized by a set of model parameter values enabling simulating the observed phenotype. Model parameters are here considered as phenotypic traits more simple (less integrative than related measured variables) and less prone to GxE. They can be used in the context of genetic studies (association mapping, QTL analysis).

EcoMeristem was initially created to address the **vegetative morphogenesis.** The model was recently adapted to deal with rice phenotypic plasticity under drought, key constraint to account for within climate change studies (Luquet *et al.,* 2006). Studies underway on rice response to extreme temperatures and atmospheric CO2 concentrations will be also used to adapt the model to such environmental constraints. Moreover, *Ecomeristem* concepts are currently extended to the **whole plant cycle to deal with the impact of climate change on reproductive morphogenesis and final yield**.

Objectives of my internship

Now the *EcoMeristem* model works well during the vegetative phase and needs to be developed for the **reproductive phase**. This period is characterized by additional demand from new sinks like **internode elongation**, carbohydrate **reserve accumulation** and remobilization from internodes and **panicle development**, while demands from other sinks like leaf elongation and tiller creation will stop. In order to integrate the demand of these new sinks and reduce gradually the demand from leaf sinks, it is necessary to supply the model with the **framework and coordination of the morphogenesis elongation of all organs with time.** This will allow the competition index used in the model to integrate the overall demand at a time, to regulate the demands in case of a nutritional deficit and to allocate newly gained biomass to the priority sinks and finally to stop tillering.

Such developmental framework will also provide a tool to predict the effect on organ size and on final leaf number of an **abiotic stress**, such as **drought**, **submergence** or **heat**, with respect to the timing of occurrence of this stress.

Thus the overall objective of this study was to understand the **rice plant development scheme** by observing the sequence of events during growth. Hence this work aims at establishing the framework with time of the elongation and increase in biomass of internodes and panicle, and to a minor degree the elongation of blades and sheaths, of **distinct tillers** of the plant until maturity, and its connection with **leaf emergence dynamics** and its variation with **contrasted varieties** and **contrasted plant densities**.

2. MATERIALS AND METHODS

2.1. Plant material and growing conditions

Two trials were conducted in IRRI field experimental station during the 2010 dry season (January-May).

In trial 1, **three genotypes** were studied: **IR64, SACG-7** and **NPT.**
IR64 is a high-tillering semi-dwarf plant with small thin leaves.
SACG-7 is a low-tillering tall plant with long large leaves.
NPT is a low-tillering intermediate height plant with thick organs.
All are indica type varieties.
The seeds of IR64 and SACG7 were sown on 16 January and transplanted 10 days later, i.e. on 26 January, at 20 x 20 cm spacing in the main field with 1 seedling per hill. The seeds of NPT were grown 11 days later because of the delay in their acquisition. Hence they were sown on 27 January and transplanted on 5 February at the same density of population than IR64 and SACG7.

In trial 2, **two densities** were compared: **12.5 plants.m^{-2} (**20 x 40 cm spacing) and **50 plants.m^{-2}** (20 x 10 cm spacing). The genotype used was **IR72** (which is also an indica rice cultivar).
The sowing occurred on 1 March and the transplanting occurred 10 days later, i.e. on 11 March, in the main field with also 1 seedling per hill.

Each of the 5 plots (IR64, SACG7, NPT, IR72 Low Density, IR72 High Density) was 12 m wide and 20 m long (Figure 1).

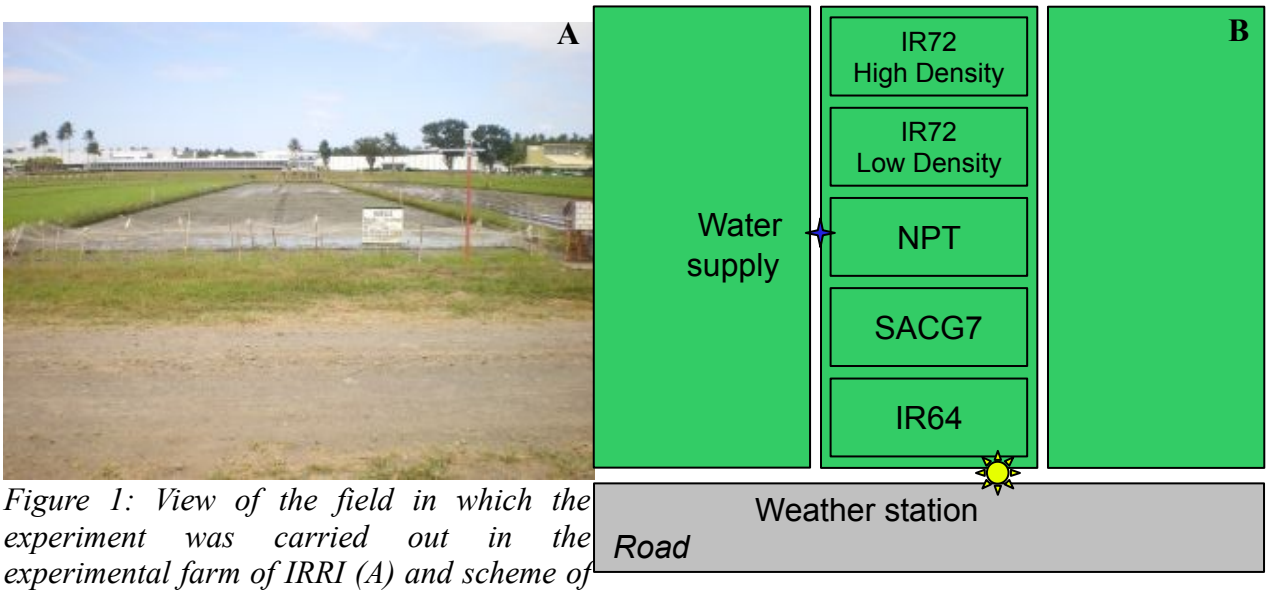


Figure 1: View of the field in which the experiment was carried out in the experimental farm of IRRI (A) and scheme of the different plots inside the field (B)

All plants received optimal N management: urea was applied with the rate of 130 kg N/ha (basal application of 40 kg, and 3 splits with 30 kg per split). P was applied at basal using solophos at 40 kg P/ha, K as muriate of potash at 40 kg K/ha rate and zinc at 5kg Zn/ha using zinc sulfate.

Molluscicides was applied to control snails before and after transplanting. Cymbush was sprayed to control insects. Furadan granules were applied to control maggots at the early vegetative phase and before the reproductive phase to control stem borer. No Tungro was observed, but we observed white heads due to stem borers.

Flooded condition were always maintained at 3 to 5 cm standing water above soil surface.

The same measurements were made on the two trials. However, trial 1 was carried out from transplanting until maturity whereas trial 2 could only be carried out from transplanting until flowering.

2.2. Weather data

Radiation, Air Temperature and Relative Humidity were recorded every 30 minutes using a weather station next to the field.

Soil temperature was also measured using 32 thermocouples located in different places in the field.

Most results in this report will be presented in chart with **thermal time** (in degree day °Cd) on the x-axis which takes climate conditions in account. Thermal time was calculated from sowing, using the every day mean of air temperature and considering a base temperature of 12°C. However, the temperature in Los Banos was rather constant thus we have almost a linear relation between time and thermal time (Figure 2).

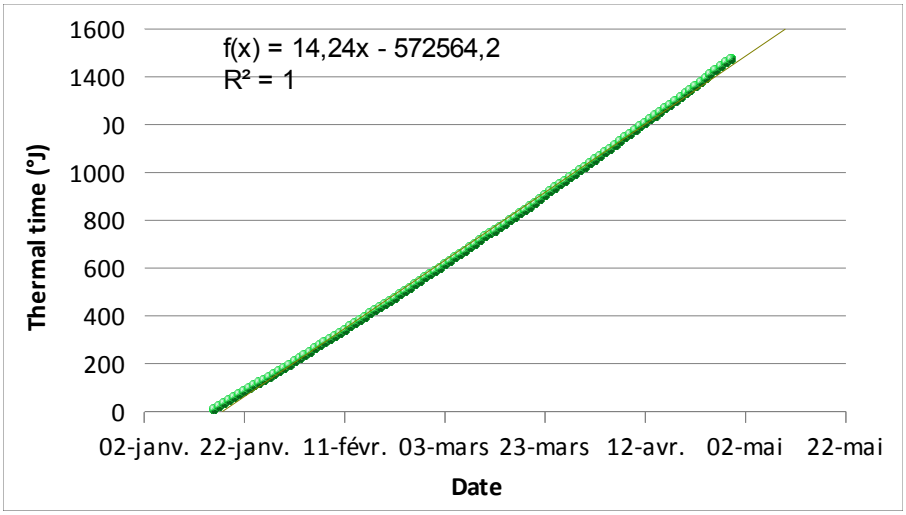


Figure 2: Linearity between thermal time (in °Cd) and time

2.3. Graphical and statistical analysis

Graphs presented in the report were made with Microsoft Excel. To characterize the deviation of the mean, the standard error (SE) was used:

SE = Standard deviation / square root of the sample size.

Statistical analysis was made with XLSTAT. Usually we used a significance level of 0,01.

2.4. Regular non destructive plant measurements

Twice a week from transplanting until panicle initiation (PI), and once a week from PI until maturity, non destructive measurements were carried out on four repetitions of eight plants each. Each repetition was located on one of the four sides of the field (Figure 3).

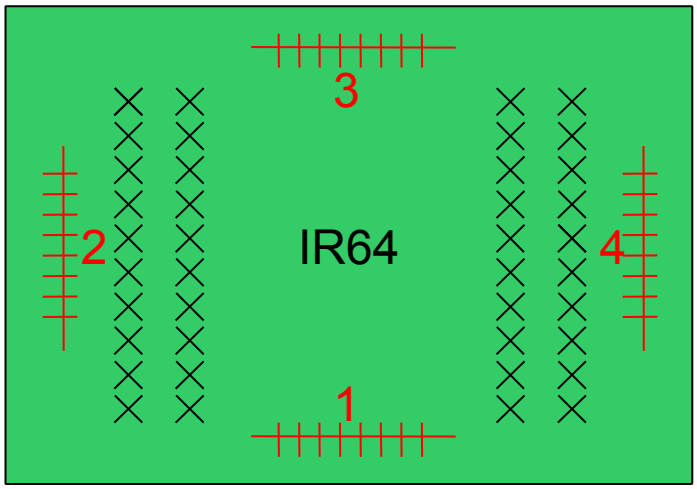


Figure 3: Position of the plants studied inside each plot. In red the position of the 4 reps used in non destructive measurements. Black crosses represent the places where sets of 4 (or 6) plants where sampled for regular and specific destructive measurements.

First, the number of visible tillers on each plant was counted.

Then, observations were made specifically on three tillers of each plant: the main tiller (MT), the primary tiller emerging from the 4th leaf of the MT (T4) and the secondary tiller emerging from the 2nd leaf of T4 (T4-2). Usually, T4 was the first or second tiller emerging on the plant from the MT. This tiller was always present on the plant. This choice gave us an idea of the features of the tillers that we expected to be the two most productive tillers on the plant: MT and T4. T4-2 was not always present on the plant, hence it gave us an idea of the rate of senescence and the features of a secondary tiller. These specific tillers were recognized thanks to coloured rings put in specific leaves at a early stage of growth and regularly updated.

On these three tillers on each plant, the number of green leaves, of visible leaves (number of the last emerged leaf) and of visible collars (number of the last leaf with a visible collar) were determined.

To be more precise, the number of visible leaves was decimalized using the length of the emerged part of the last blade compared to the length of the penultimate blade (Annex 2). The number of visible collars was decimalized using the angle between the last two blades (Annex 3).

2.5. Regular destructive plant measurements

Three times a week from transplanting until booting, and then once or twice a week from booting until maturity, destructive measurements were carried out on four repetitions of one plant each (each individual plants sampled was considered as a repetition). These samples were taken from plots of four plants that had been selected randomly in the field (Figure 3) and tagged previously (Picture 2).

Picture 2: Tagging in the field with colored rings to distinguish the different leaves (and later on the different tillers)

The same phenological observations than in non destructive measurements were made (number of visible tillers of the whole plant, number of visible leaves, green leaves and collars of MT, T4 and T4-2). Besides, the length of the blade and the sheath of each phytomer was measured using a ruler and a binocular microscope for the smallest leaves. The length and diameter of each internode were also measured using a ruler and a Caliper respectively when the internodes became clearly visible (more than one millimeter in length). The length of the panicle was measured from panicle initiation using a binocular microscope and then using a ruler.

2.6. Specific destructive plant measurements

At specific dates and phenological stages (see Annex 1), specific destructive plant measurements were carried out on 4 or 6 plants. This allowed to get data about every two weeks.

At **transplanting**, **34 DAS** (days after sowing), **PI** and **booting** (actually, this sampling was done before booting because booting had not occurred yet two weeks after PI), we processed 4 repetitions of 1 plant each.

At **flowering** and **mid grain filling**, we used 6 repetitions of 1 plant each.

At **maturity**, we used 3 repetitions of 4 plants each (sampled in the middle of the field).

At those stages, we did the same measurements that we did at regular destructive plant measurements. We also measured the width of the blades, the leaf area of the last expanded leaf (the last leaf with a visible collar) of each specific tiller and the leaf area of the other leaves. The dry weight of each part (blades, sheaths, stabble, internodes, panicle, dead leaves) was also measured on each specific tiller and on all other tillers together. The number of spikelets was measured at flowering and maturity on all panicle of the plant.

Moreover, at PI, flowering, mid grain filling and maturity, we measured the length of the culm (sum of internodes) of all tillers on the plant.

3. RESULTS

Because of the amount of data collected, it was not possible to encode and analyze all of them during the period of my internship. Hence these results concern only the first two genotypes grown: **IR64 and SACG7**.

First will be compared the results obtained on these two genotypes, then we will compare the three tillers observed on each genotype. Finally, the coordination of the events in the growth of the rice plant will be studied: at the phytomer level, at the tiller level, and at last at the whole plant level.

3.1. Comparison of two contrasted genotypes: IR64 and SACG7

3.1.1. Tillering and tiller senescence

The evolution of the number of tillers through non destructive measurements on 4 reps of 8 plants per genotype from transplanting until maturity is shown in Figure 4. These data are compared with those collected through regular destructive measurements, involving 4 plants at each date. The set of 4 plants was sampled randomly in a different place in the field each time. That is why the destructive dynamic is irregular, compared to the regularity of the non destructive dynamic which has been done with the mean of the same 32 plants during the whole experiment. The discrepancies between both dynamics (the tiller number measured from destructive was consistently higher than that collected from non destructive measurements) can be explained by the fact that all tillers of the plant can be observed and numbered while dissecting the plant in the lab whereas some very small tillers might be not seen when counting them in the field.

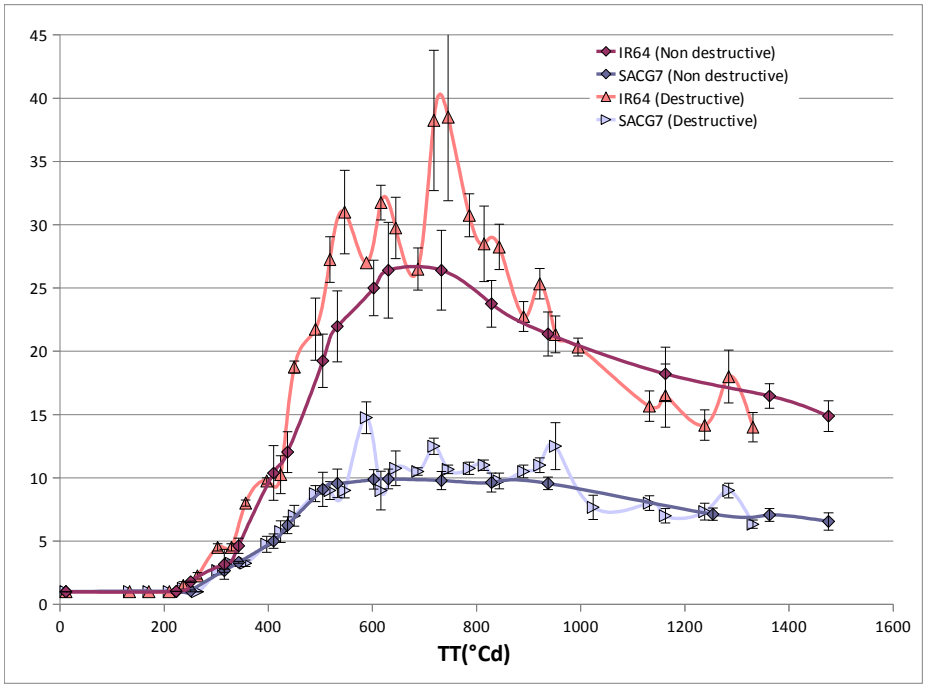


Figure 4: Evolution of the number of tillers for IR64 and SACG7. Vertical bars represent standard errors.

These observations confirm that IR64 is a high-tillering genotype. Indeed, its average maximum number of tillers was 38,50 for destructive (the highest value among the 4 plants was 58) and 26,41 for non destructive (the highest value among the 32 plants was 37). Its average final number of tillers (at maturity) was 16,00 for destructive and 14,88 for non destructive. On the other hand, SACG7 is a low-tillering genotype with a maximum number of tillers of 14,65 (the highest value among the 4 plants was 18) for destructive and 9,91 for non destructive (the highest value among the 32 plants was 15), and an average final number of tillers of 6,42 for destructive and 6,55 for non destructive. This difference of tillering is also obvious in the field (Picture 3).

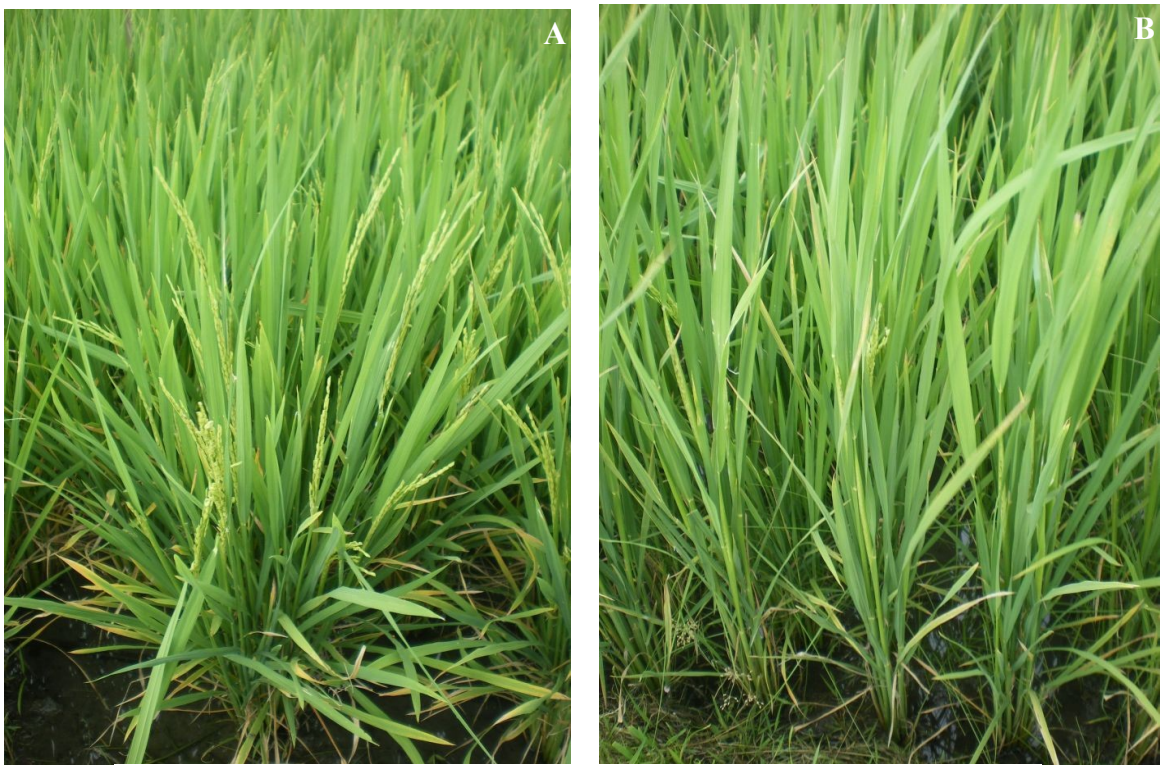
We can observe that the final number of tillers is equivalent to the number of tillers around 430°Cd for both genotypes. Moreover, both reach their maximum tillering around 680°Cd.

Thanks to non destructive data, we can calculate an approximate rate of tiller senescence for each genotype:
R = (maximum number of tillers - final number of tillers) / maximum number of tillers x 100

Hence, R_{IR64} = 52 % and R_{SACG7} = 53 %.

Then we can consider that less of half of the tillers produced grain while the remaining died.

According to our destructive and non destructive data at maturity, we noticed that the primary tiller T4 was always present on the plant, except on 2,27% of IR64 plants, and 6,82% of SACG7 plants. Nevertheless, the senescence (or the non birth) of the secondary tiller T4-2 was much higher: 13,64% for IR64 and 61,36% for SACG7. Thanks to non destructive follow-up, we know that the absence of those tillers at maturity was always due to the senescence of previously emerged tillers for IR64 whereas sometimes it concerned plants in which tiller T4-2 never emerged (in 42,11% of cases).



Picture 3: Difference of tillering of IR64 (A) and SACG7 (B) at flowering stage

3.1.2. Yield

	IR64		SACG7	
	Mean	Standard error	**Mean**	Standard error
Panicles per plant	**16,00**	0,84	**6,42**	0,40
Spikelet number per plant	**976,58**	70,27	**665,83**	52,56
Spikelet number per panicle	**78,11**	1,99	**178,35**	7,74
Filled grain percentage	**85,40%**	1,26%	**74,15%**	2,63%
Individual grain weight (g)	**0,029**	0,001	**0,029**	0,001
Panicle weight (g)	**1,797**	0,064	**3,747**	0,190
Weight of all panicles of one plant (g)	**29,43g**	1,89g	**24,00g**	1,60g

Table 1: Yield components at maturity for IR64 and SACG7. These data come from the 3 reps of 4 plants dissected at maturity.

Even if IR64 produces much more panicles per plant than SACG7, the average weight of all panicles of one plant at maturity was not that different between the two genotypes (29,43g for IR64 and 24,00g for SACG7). This is due to the fact that SACG7 compensate with the number of spikelets per panicle (SACG7 has more than twice as many spikelets per panicle as IR64). Moreover, the filled grain percentage are quite close between the two genotypes, and the individual grain weight is the same for both.

This expresses a different strategy of these two genotypes to produce grains: one prioritized the panicle number per plant while the other prioritized the grain number per panicle. For all that, IR64 yield per plant (and also the yield per hectare, because the density was the same for both genotypes) was 23% higher than SACG7 yield.

3.2. Comparison of different tillers of the plant

3.2.1. Position of the chosen tillers among population

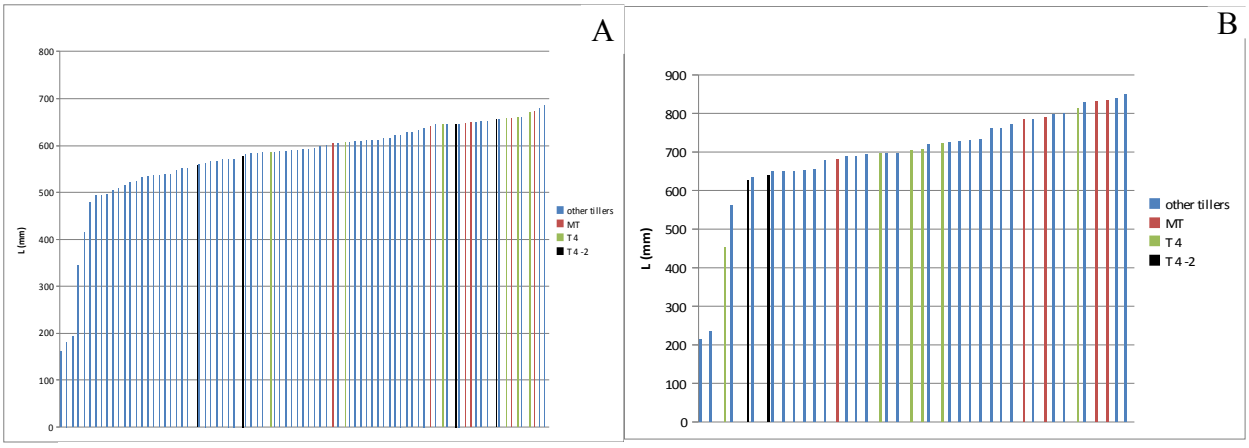


Figure 5: Length of all culms of IR64 (A) and SACG7 (B) plants at mid grain filling stage. The culm length is calculated as the sum of internodes lengths. For each genotype, the culms of 6 plants are considered.

At mid grain filling, the culm has already reached its final height. For SACG7, the main tiller is among the highest tillers, the tiller T4 is in the middle and the tiller T4-2 is among the shortest tillers. For IR64, the tiller T4 is often higher than the main tiller ; both are among the highest tillers. The tiller T4-2 has a wide dispersion ; it can be in the middle or among the highest tillers but is never among the shortest.

As we expected, T4 can be as or more vigorous than MT. Nevertheless, the position in height of T4-2 among all tillers of the plants is difficult to generalize.

3.2.2. Rhythm of leaf emergence

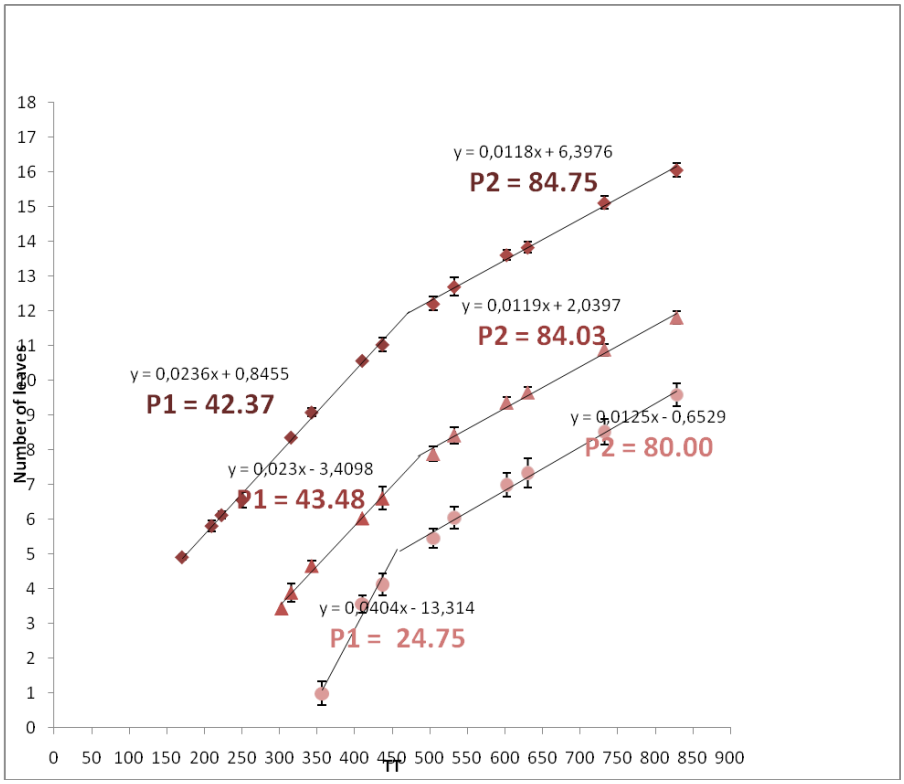


Figure 6: Evolution of the number of visible leaves on MT, T4 and T4-2, for IR64. The data come from non destructive measurements. Next to each slope, the equation of the chart, the regression coefficient (R^2), and the phyllochron are written.

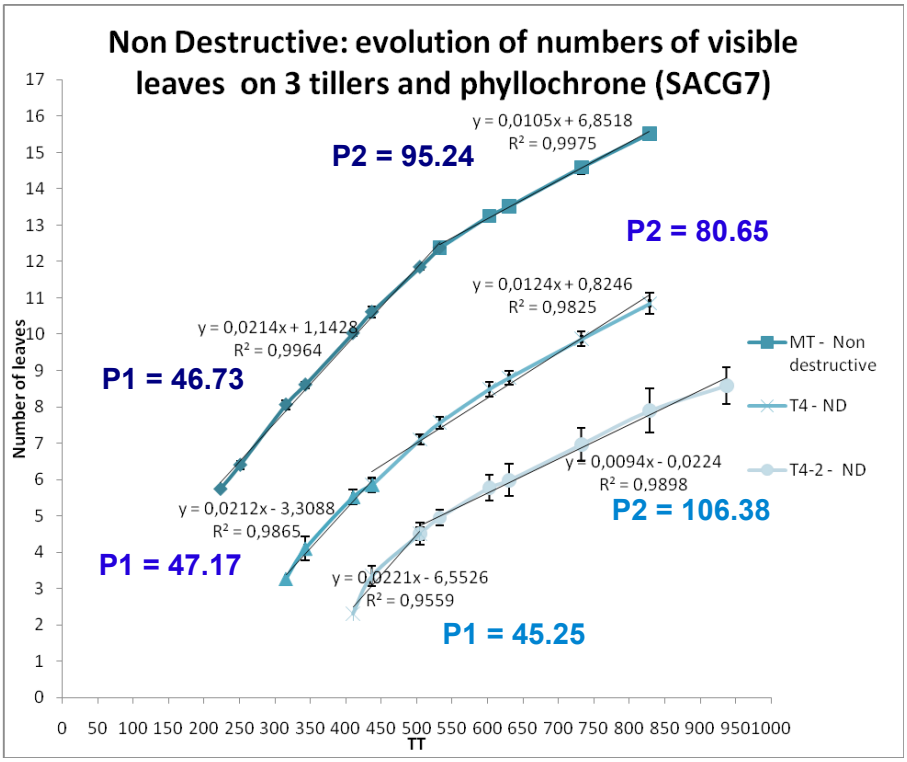


Figure 7: Evolution of the number of visible leaves on MT, T4 and T4-2, for SACG7

These charts represent the number of visible leaves on MT, T4 and T4-2, observed in non destructive measurements, until the final number of leaves is reached. For all tillers and both genotypes, the dynamics of leaf number can be fitted with two successive linear phases of growth, the slope of the second one being lower than that of the first one. This break in slope (determined statistically for IR64 and by hand for SACG7, because of a lack of time) occurred around 470°Cd for both genotypes and all tillers.

Thanks to the equation of the the chart, the phyllochron was calculated (the reciprocal of the slope coefficient) which is the period of time (in °Cd) between the emergence of two following leaves.

We can notice that they are almost the same for the different tillers of one genotype (nevertheless, we didn't have enough time to confirm this statement statistically). Basically, one leaf appears every 43°Cd on MT and T4 of an IR64 plant during phase 1. Leaves take almost twice as long to emerge during phase 2. The rhythm of leaf emergence is a bit faster on T4-2 during the two phases. On SACG7, leaves need a little bit more time to emerge compared to IR64 but the rhythm is nearly the same on the three tillers. Each leaf needs 46°Cd to emerge during phase 1 and more than twice as long (94°Cd) during phase 2.

The change in phyllochron occurs around 470°Cd on all tillers. At that moment, the rate of leaf emergence is divided by two.

Thus, even if these two genotypes have a very different dynamic of tillering, the dynamic of leaf emergence is the same.

3.2.3. Panicle initiation and elongation

During destructive measurements, the initiation of the panicle was observed using binocular microscope observation. We noticed that there was no order in panicle initiation among tillers. Sometimes the panicle was only visible on one tiller among MT, T4 and T4-2. Sometimes it was visible on two of them. Sometimes a panicle shorter than 1 mm was observed at the same time on the three tillers. We can assume that in that case the panicles were nearly initiated at the same time on all tillers.

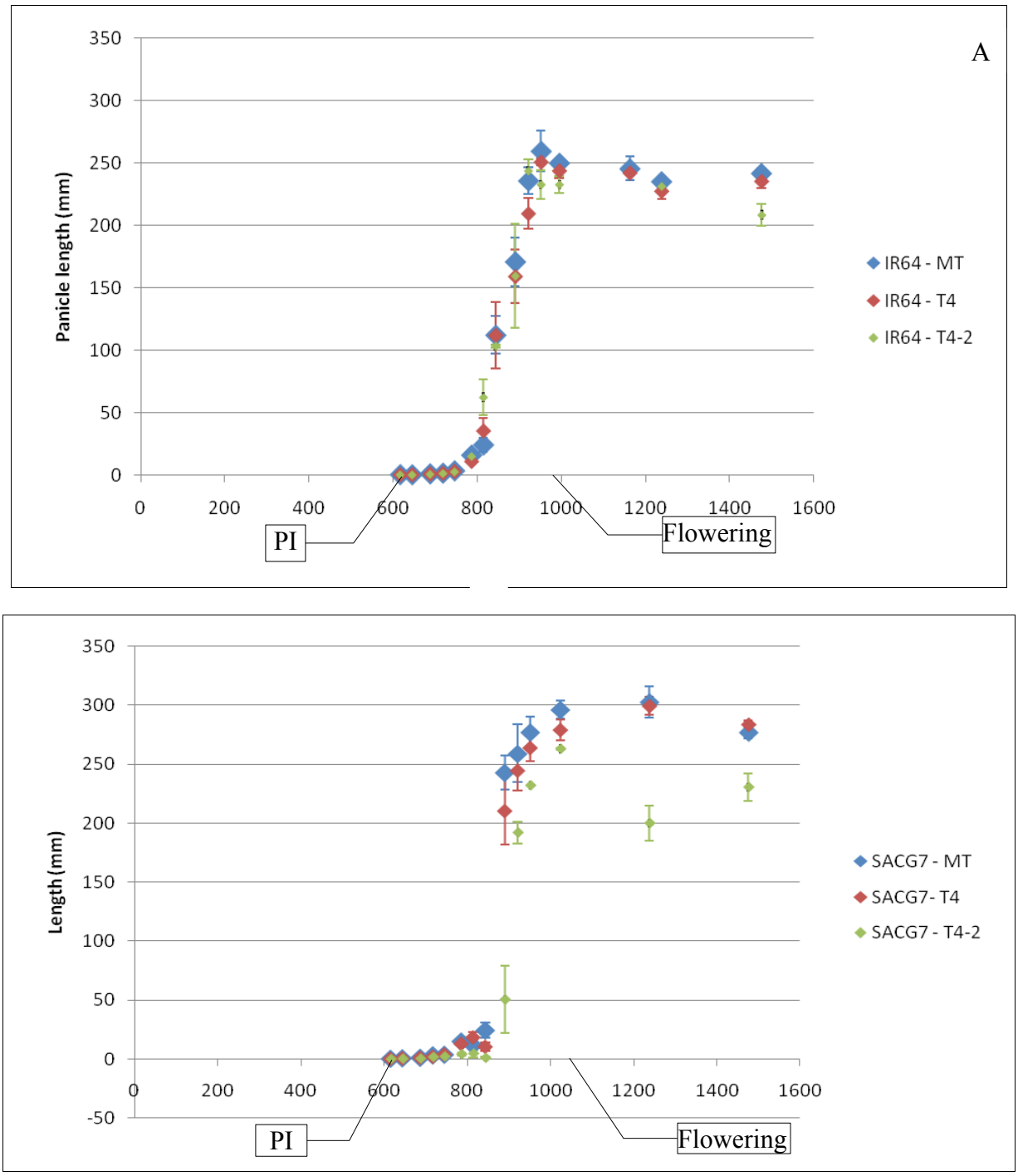
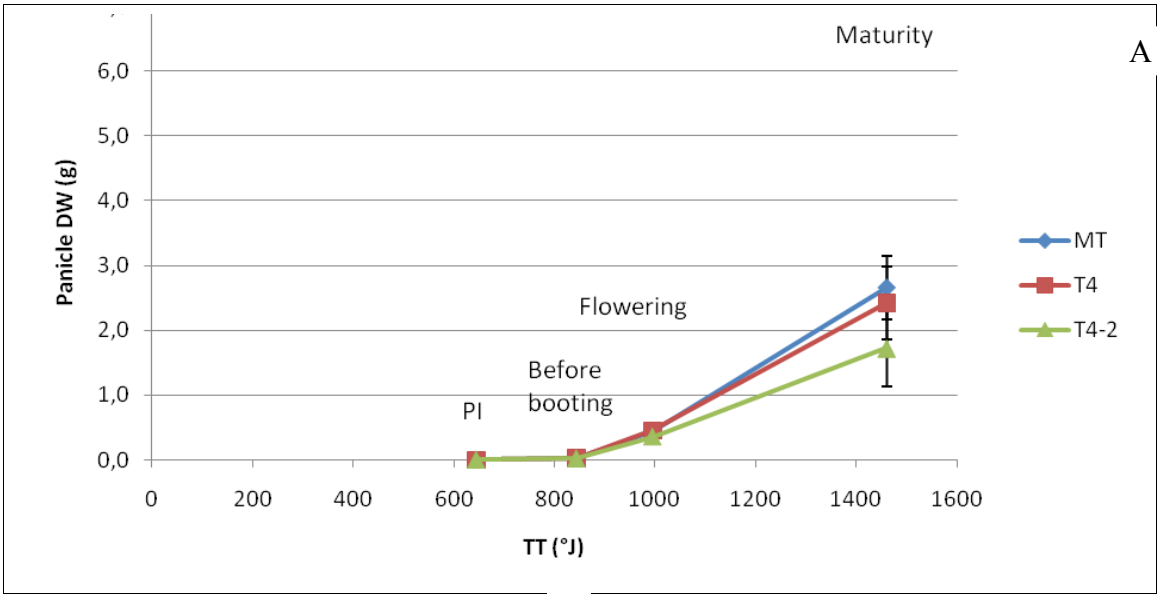


Figure 8: Panicle length of the three tillers of IR64 (A) and SACG7 (B)

For IR64, the growth of the panicles between initiation and heading seemed to be synchronous whatever the tiller origin was until they reach their final mean length of 240 mm around 920°Cd. Yet a Tukey test (HSD) with the data at maturity concludes that T4-2 panicle length was significantly shorter than that of MT and T4. It is important to keep in mind that only panicle length is considered here and not panicle biomass. For SACG7, the panicles of MT and T4 elongate almost identically and reach mean length of 280 mm around 920°Cd whereas the panicle of T4-2 remains smaller : 230 mm in mean. The Tukey test confirms this observation. Even though the panicle of T4-2 in SACG7 was finally smaller than those of MT and T4, it is important to notice that its elongation rate appeared to be similar to that of the 2 other tillers. The smaller final size of the panicle T4-2 appeared to be the consequence of an earlier cessation of growth rather than to a slower elongation rate.

3.2.4. Yield



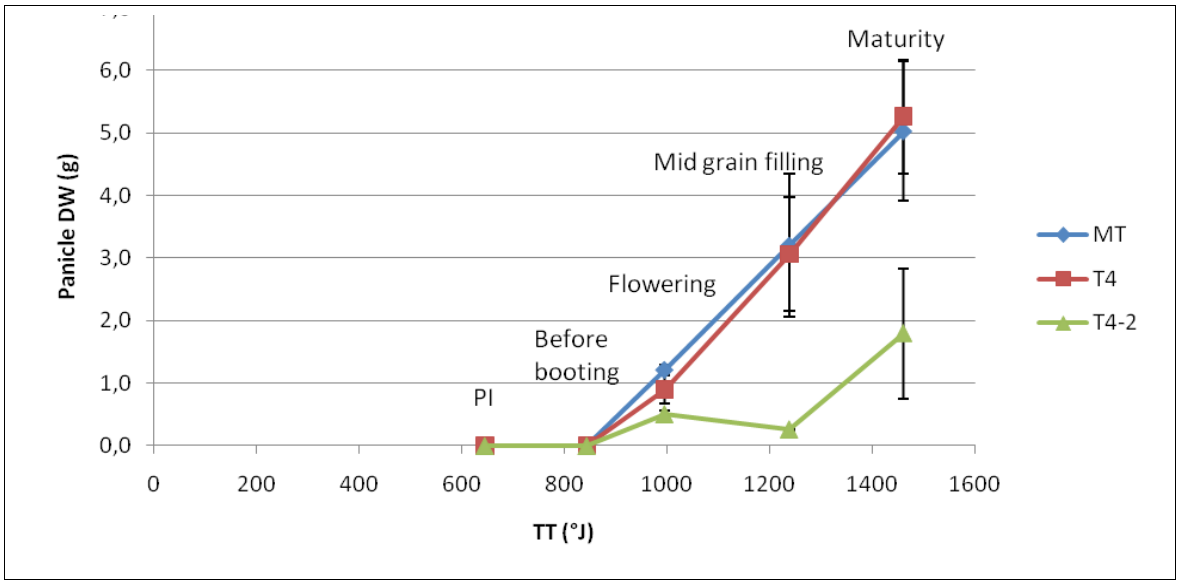


Figure 9: Panicle dry weight on the three tillers of IR64 (A) and SACG7 (B), measured at panicle initiation (PI), before booting, at flowering, at mid grain filling (for SACG7 only) and at maturity

The panicles start gaining weight at panicle initiation (Figure 9), yet their growth in dry weight remains very slow until booting. At booting, around 840°Cd, while their height is one third of their final height for IR64 and less than one tenth for SACG7, the growth in dry weight become faster. Then , panicle growth seemed to be linear. At flowering, they weigh one fifth of their final weight while they are already fully extended (for both genotypes). We notice a significant difference in final biomass between MT and T4 panicles and T4-2 panicles. It was also confirmed by a Tukey test. This difference is specially large in the case of the few remaining tillers T4-2 of SACG7 whose panicle are three times lighter than MT and T4's.

Is this difference due to a difference in filling rate or in number of grains or in weight of one grain?

Tiller	Mean of panicle DW (g) / Number of filled grains *1000	SE
MT	26,39	1,96
T4	29,77	7,76
T4-2	25,51	7,02

Table 2: 1000-grain weight for the panicles of the three tillers of SACG7. The weight of unfilled grains is considered negligible compared to the weight of filled grains.

The 1000-grain weight is higher for T4 than for MT and for MT than for T4-2. However the difference doesn't seem to be significant.

Hence the difference in panicle weight can be due to a difference in the number of spikelets per panicle or a difference in the grain filling rate (number of filled grains / number of filled and unfilled grains * 100).

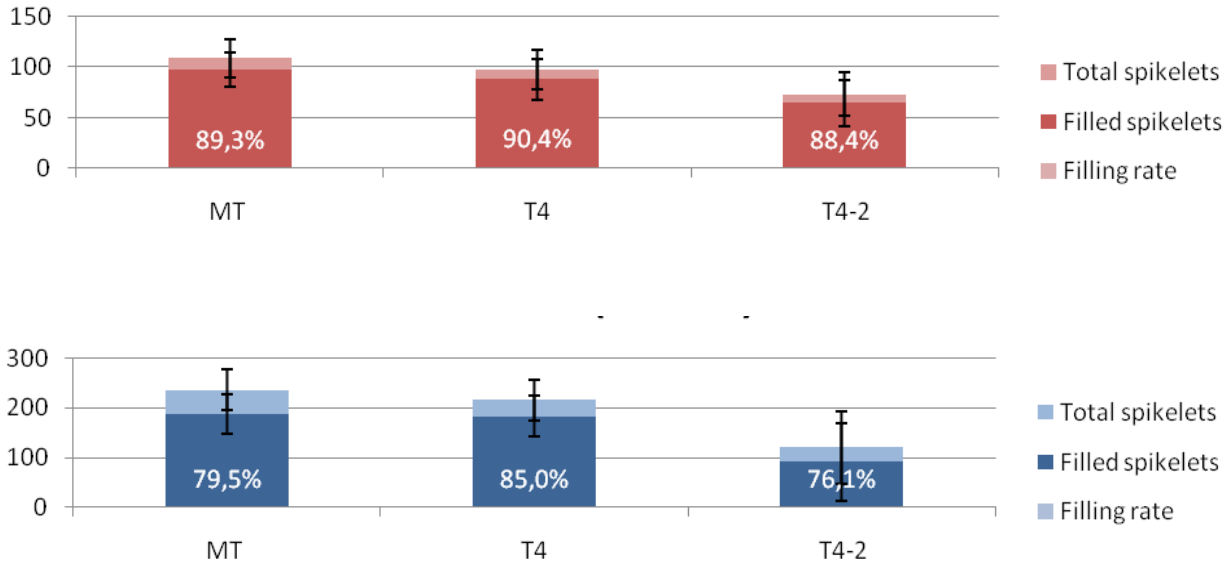


Figure 10: Number of grains per panicle at maturity on the three tillers of IR64 (A) and SACG7 (B). In light color, the total number of grains; in dark color the number of filled grains; inside the box, the filling rate in percentage.

Indeed, the grain filling rate is a bit lower in T4-2 than in MT or T4 for both genotypes (Figure 10), but the difference is minimal. On the other hand, we see on the charts that T4-2 has much less spikelets per panicle than MT and T4 for SACG7. This is the main explanation of its low biomass.
Thus, the difference of potentiality between tillers according to their initiation date is mainly due to their number of spikelets per panicle.

3.3. Correlation and coordination of the events in the growth of the rice plant

3.3.1. Coordination inside one phytomer

We numbered internodes according to the phytomer they belong to. A phytomer consists of a leaf (blade and sheath), an internode, a node and an axillary bud. For example, the internode 12 is the one belonging to the phytomer 12 ; it is located just below the sheath of the leaf 12. We considered the peduncle (that bears the

panicle) as the last internode.

Thanks to regular destructive datas, the increase in length of the different organs of each phytomer is known. As an example, the elongation of the organs of phytomer 15 is presented in Figure 11.

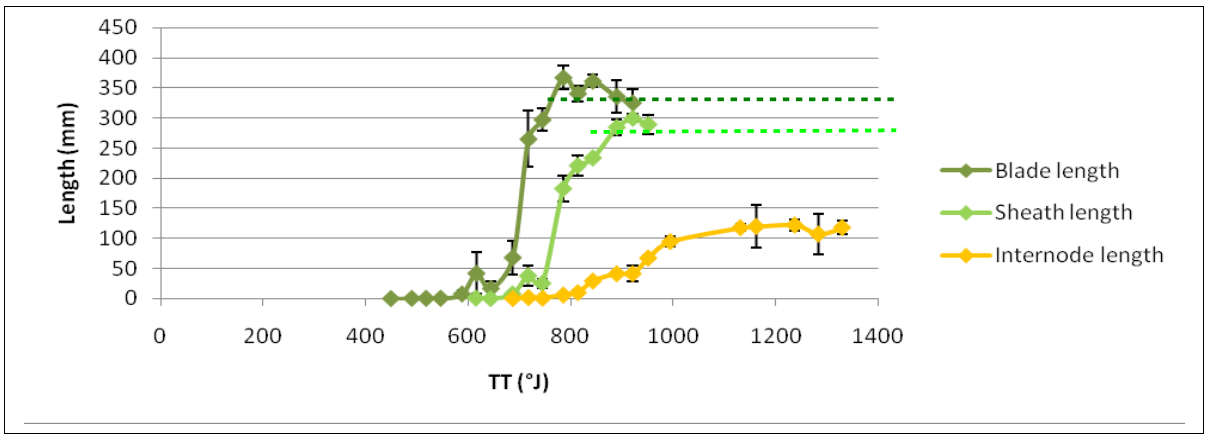


Figure 11: Elongation of blade, sheath and internode of the 15th phytomer of the main tiller of IR64. Dotted lines indicates the final length of blade and sheath which have not been measured once they were considered fully extended.

Both blade and sheath follow a development scheme with a slow phase followed by a rapid phase. The blade slow phase starts around 600°Cd. Its rapid phase starts around 700°Cd, at the same time than the sheath slow phase begins. The blade stops elongating when the sheath rapid phase begins, around 750°Cd. At that moment, the internode starts elongating. It elongates more slowly than blade and sheath. When the sheath elongation is done, around 900°Cd, the internode has just reached half of its final length. It stops elongating around 1200°Cd.

Thus, for this phytomer, the blade elongation lasted 150°Cd, that of sheath 200°Cd and that of internode more than 300°Cd. Elongation of the organs of one phytomer are successive.

3.3.2. Coordination of the growth of all the shoot organ of one single tiller

Figure 12 has been made from the analysis of the same graphs than Figure 11 for phytomers 12 to 18 (the 18th phytomer is composed of the panicle and the last internode, the pedoncle). It gives a global view of the coordination of organs growth inside one tiller. For example, we see that the panicle elongates completely before the last internode extension.

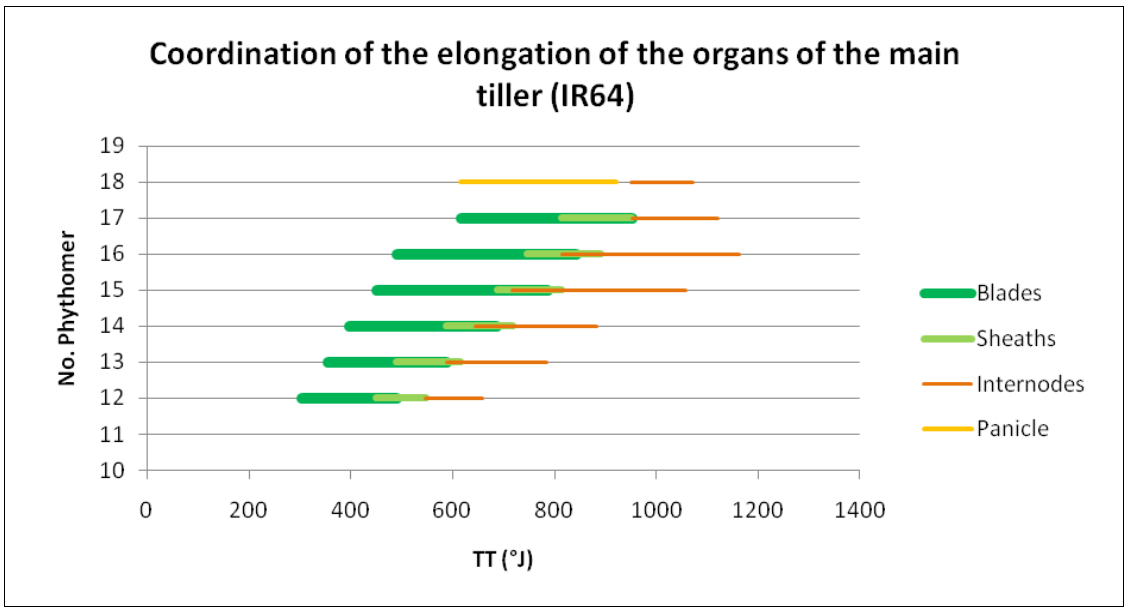
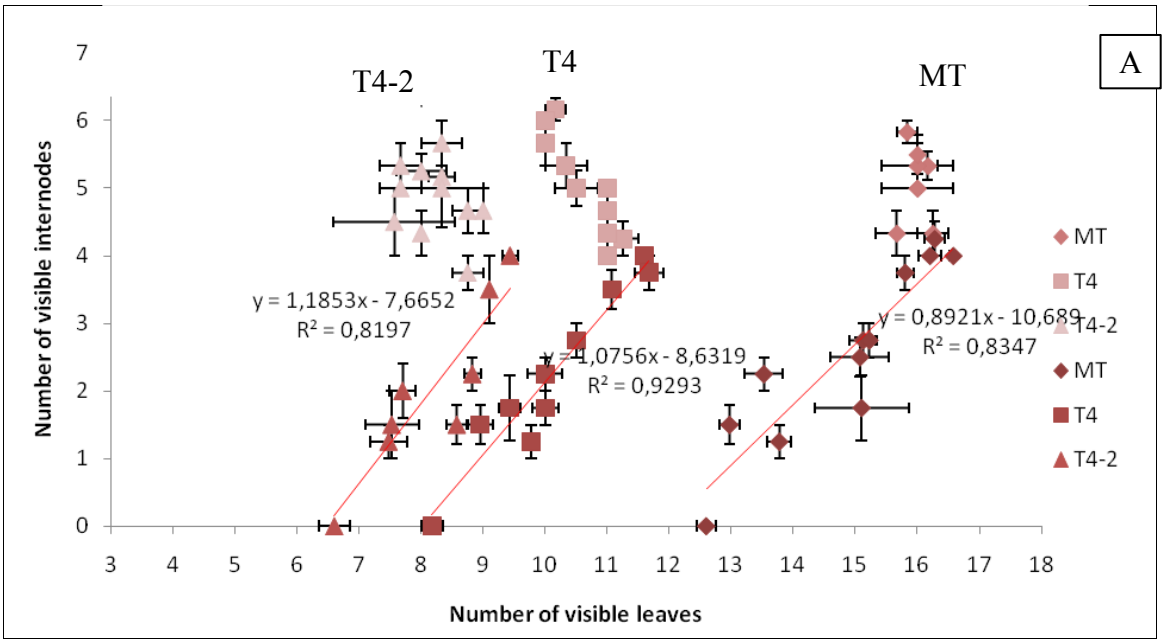


Figure 12: Thermal time courses of the organs elongation of the MT of IR64

Later graphs will present relationships between internodes and leaves and internodes and panicle.

Internodes and leaves



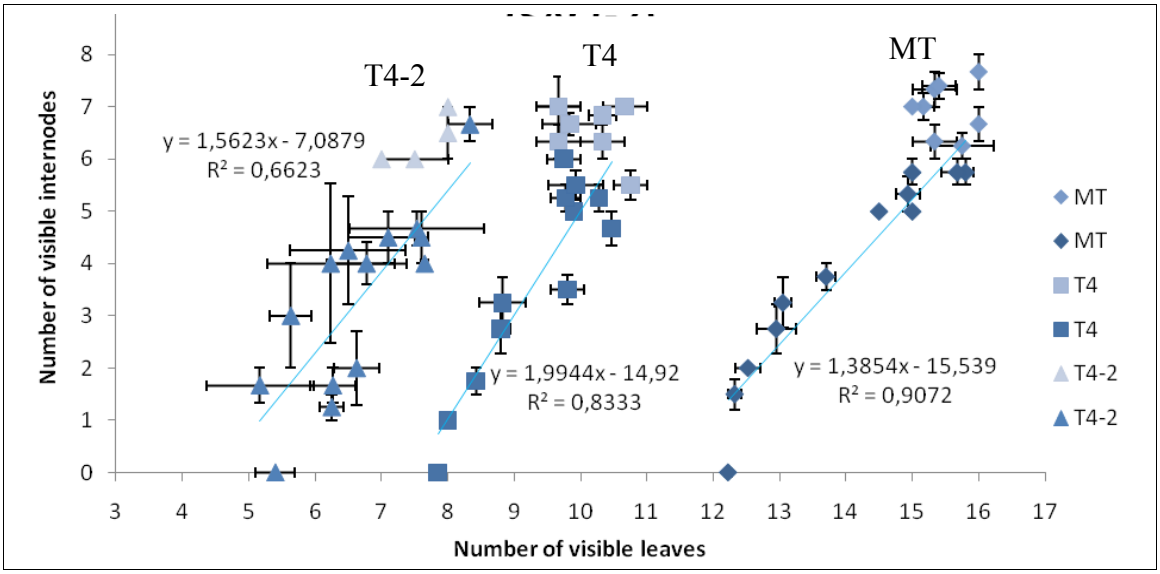


Figure 13: Number of visible internodes in function of the number of visible leaves for IR64 (A) and SACG7 (B). From the right to the left: MT, T4 and T4-2.

These graphs were created using the destructive measurements data per date, from the beginning of elongation of internodes until the moment when all leaves have emerged (in dark colours) and from that moment until the end of elongation of internodes (in light colours). An internode is considered visible once it is more than 1 mm long. Hence the number of visible tillers includes both elongating internodes and already fully elongated internodes.

First, the slopes are almost the same for the different tillers of IR64 (but it is not the case for SACG7). However, the number of visible internodes increases faster in SACG7 than in IR64. For example, with tiller T4 of SACG7, the number of visible internodes increased by 2 when at the same time the number of visible leaves increased only by 1. On tillers T4 of IR64, only one internode starts elongating while one leaf is emerging.

After all leaves have emerged, two or three internodes more elongate on IR64 tillers whereas only one or two will elongate on SACG7 tillers.

According to the standard error bars, we observe once again much more dispersion for tiller T4-2 than for other tillers.

Internodes and panicle

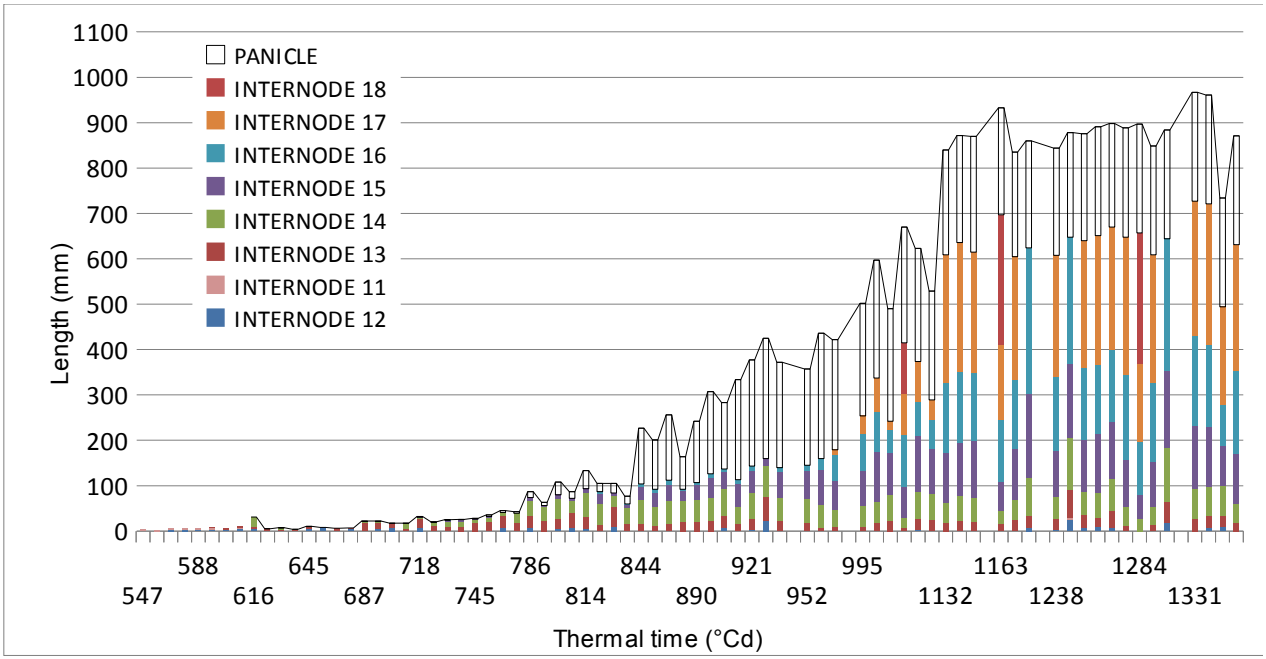


Figure 14: Elongation of internodes on the main tiller of IR64. Each stick represents one plant.

The panicle appears around 600°Cd on the main tiller of IR64, while two or three internodes a few millimeters long are visible. The panicle elongation occurs mainly between 780 and 920°Cd. When the elongation of the panicle is done, the culm is around 150 mm long (¼ of its final length) and consists of four internodes (usually internodes 12 to 15). Then two or three internodes more, the longest ones, will appear.

Despite differences in total leaf number, the phenology was the same within one tiller type: despite the peduncle of the MT of IR64 was in some cases internode 16 and in other case internode 18, it elongated at the same time.

We made the same observations on the other tillers of IR64 and on SACG7 tillers.

3.3.3. Correlation between panicle dry weight and other characteristics

- ***At maturity***

IR64:
Using destructive measurements at maturity, correlations we studied between panicle DW, number of spikelets, panicle length, flag leaf length, culm length and number of visible leaves on 12 plants.

At first, we studied the correlations between these characteristics using the data from MT, T4 and T4-2 all together. All characteristics were significantly (P<0,01) and positively correlated together. Nevertheless, these good correlations could have been due to a strong difference between, on the one hand MT and T4 data with high values, and on the other hand T4-2 data with lower values.

Then the data from MT, T-4 and T4-2 were studied separately. With the three tillers, the panicle DW was significantly (P<0,01) correlated with the number of spikelets and the flag leaf length. There was also a good correlation between panicle DW and panicle length for MT and T4 (P<0,01), and less so for T4-2 (P<0,02). However, T4-2 was the only tiller in which panicle DW correlated with culm length. As expected, there was no correlation between panicle DW and number of visible leaves.

SACG7:
Correlations between the same characteristics were studied.
Panicle DW and number of spikelets were correlated for MT (P<0,01), and T4 (P<0,02). Panicle dry weight

was correlated with panicle length only for T4. No significant correlations were made for T4-2. Indeed, there were too few values to compare because of the senescence of this tiller.

– ***Between panicle initiation and maturity***

It would be interesting to have an idea of the yield of a plant at maturity just by observing this plant at an earlier stage.

At maturity, destructive measurements were realised on 12 of the plants that were used for non destructive measurements since transplanting. Hence we got data characterizing the same plants at different dates: number of tillers and number of visible or ligulated leaves during development and grains characteristics and dry weight data at maturity.

Correlations between data from panicle initiation date and maturity were studied. No significant correlations could have been made.

3.3.4. Coordination for the whole plant

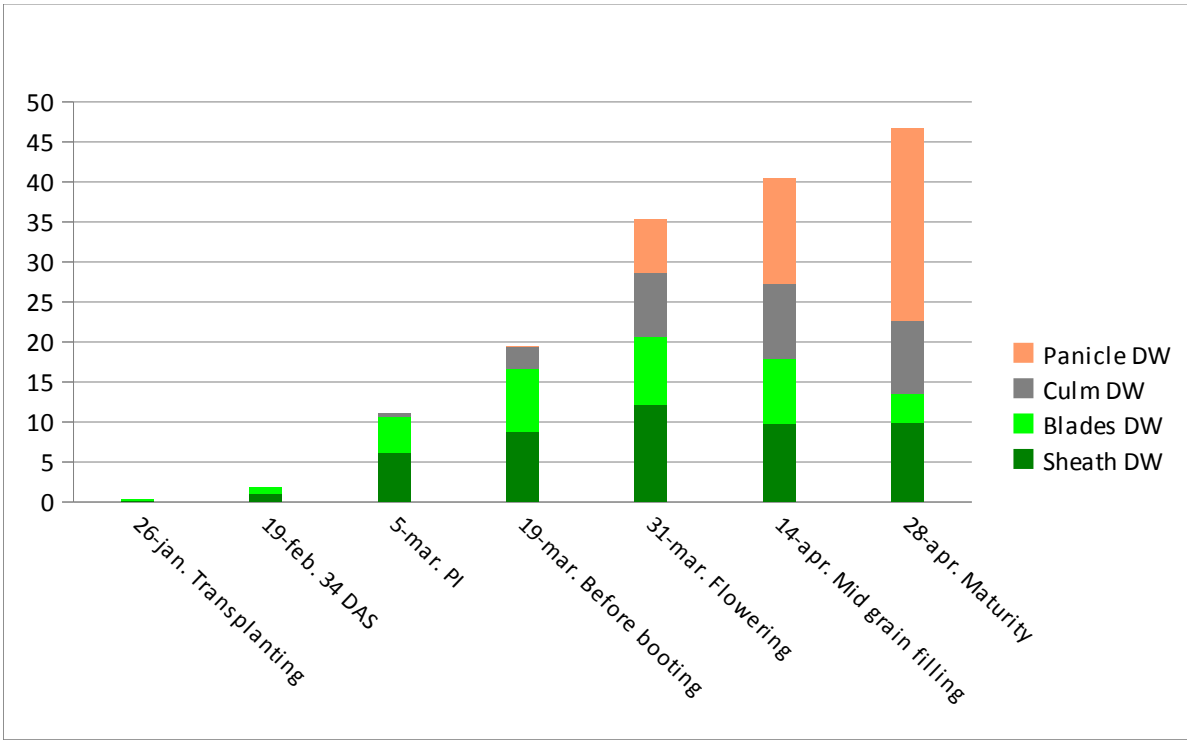


Figure 15: Evolution of the biomass distribution among the organs during the growth of the SACG7 plant. Roots were not weighed and stabble weight was neglected.

Here the evolution of the biomass distribution for the whole plant. From transplanting until booting, blades and sheath are growing and are the two main sinks of the shoot part of the plant at that stage. Then sheaths DW and above all blades DW decrease. This is due to the senescence of leaves. Culms start growing around PI and gain weight mostly between booting and flowering. Panicles really gain weight long after PI, around booting. Then they regularly gain weight until maturity. At maturity, panicles make up half of the whole plant weight.

We observe a similar evolution on IR64 plant.

The figure 16 sums up the elements of the coordination between the different events of the rice plant growth. This diagram is indicative and suitable for both IR64 and SACG7 genotypes.

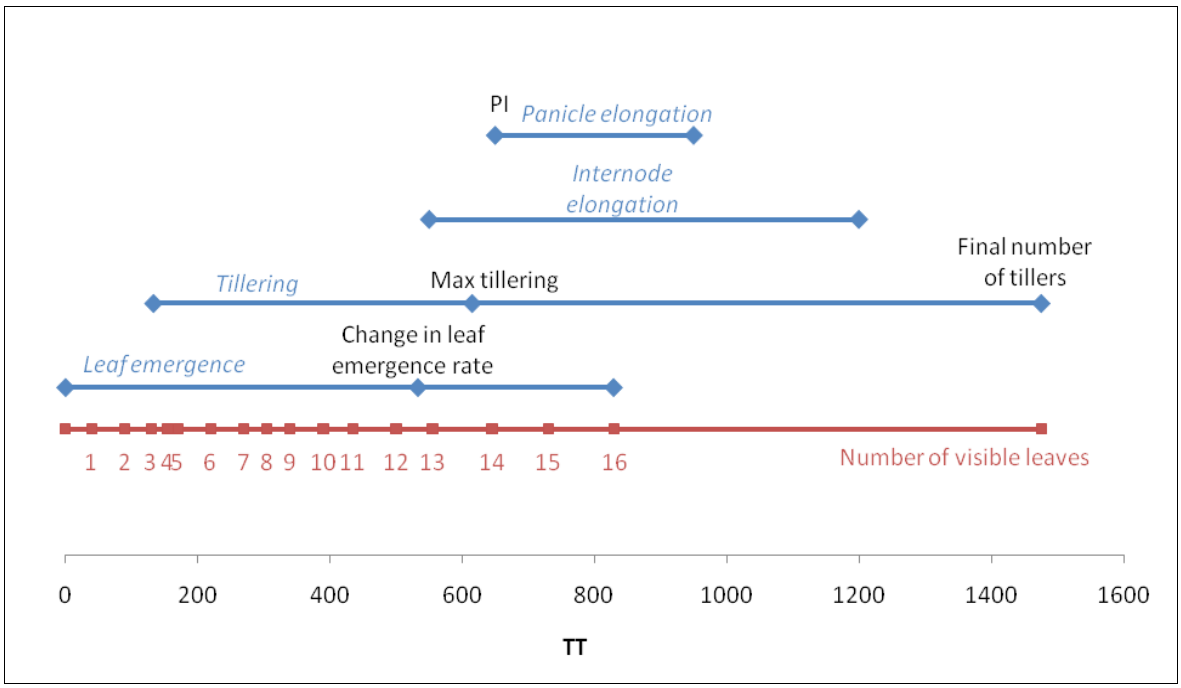


Figure 16: Coordination between the different events of the rice plant growth

4. DISCUSSION

4.1. Variability inside the field

For both genotypes, a high variability in terms of tillering was observed across sampling areas in the field. This variability was expressed between the different repetitions in non destructive measurements and from one sampling to another in destructive measurements. This can be due to variability in soil composition, texture and compaction, in land leveling and water control, in transplanting efficiency between transplanters, pests attacks like snails at very early stage.

However, there was not much difference between destructive and non destructive mean results. Hence we can assume that the plants used for non destructive measurements were not injured by frequent handling.

4.2. Variability between genotypes

IR64 and SACG7 were two genotypes interesting to compare because of their contrast in biomass accumulation and assimilates distribution. The first one gives priority to tillers creation whereas the second one favours large organs development and spikelets creation. in those conditions, IR64 was more productive than SACG7. Maybe in different conditions of temperature, radiation, humidity, etc., SACG7 could have been more productive than IR64.

Despite these differences, they have the same dynamic of leaf emergence, with close values of phyllochron. According to Khanal (2005), 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. Both genotypes were exposed to the same values of these parameters (even if there could have been small differences in soil quality, exposure to light and air, and nutrient availability). However the change of phyllochron depends on the genotype and/or the growth duration and might reflect internal or physiological changes which occur during the life cycle of rice (Itoh *et al.*, 2001).

4.2. Variability between tillers

Even if the duration of the vegetative period is shorter for the late-initiated tillers, it seems that panicle initiation occurs at the same time on all tillers of the rice plant. Synchronization was also observed for flowering and maturity (though it was not observed as precisely as PI). Then the reproductive phase, that begins at PI, has the same duration for all tillers. (This was not observed by Mohapatra *et al.* (2008) who observed only a synchronization for maturity.)

Some tillers (like T4) can reach the same yield at maturity than the main tiller. However some tillers (like T4-2) have a yield lower than the main tiller, because of a lower number of spikelets per panicle. This number of spikelets, the sink size, is established at flowering (Lafarge *et al.*, 2009), hence the the difference of yield potentiality between tillers can be known at flowering.

4.3. Leaves and internodes extension

Both blade and sheath follow an extension scheme with a slow phase followed by a rapid phase. It is probably an exponential phase followed by a linear phase, like Lafarge and Tardieu (2002) demonstrated in sorghum.

Almost the same framework was used to describe internode extension in maize (Fournier and Andrieu, 2000; Birch and al., 2002). Four phases were described: an initial exponential stage, transition to rapid extension, rapid (linear) extension and transition to final length.

Our measurements on rice are not precise enough to mark out such phases, but we can suppose that internode

extension in rice is similar to that in maize.

4.4. Coordination of the events in the growth of the rice plant

According to Takeda (1977), the panicle formation is a trigger for starting internode elongation in rice plants with a small number of nodes on the main culm but not in rice plants with a large number of nodes. IR64 and SACG7 have a medium number of nodes. We observed that the panicle was usually initiated when some short internodes were already visible. Hence both genotypes can be considered as part of an intermediate group in Takeda's description. Perhaps first internodes appear before panicle initiation but start their rapid extension phase once the panicle is initiated. Further and more precise experiments would be necessary to demonstrate this.

This moment is an important transition in the dynamic of biomass distribution in the plant. Indeed, around PI, the rhythm of leaf emergence and the number of tillers decrease, while internodes and panicle start elongating.

5. CONCLUSION AND PERSPECTIVES

This study provides precise data about the number, the size and the biomass of the different elements (tillers) and organs (leaves, internodes, panicles) of the rice plant for two genotypes (IR64 and SACG7) at several dates and stages. It specifies the framework and coordination of the morphogenesis elongation of these organs. These two contrasted genotypes were chosen to provide a wide data range.

Some of these data will be useful to improve the model *EcoMeristem* for the vegetative phase (phyllochron values, dynamic of tillering...) and more specifically to develop it for the reproductive phase (internodes growth in length and diameter, panicle development, coordination of these events in one tiller, coordination of these events between tillers in one plant...).

Thus, this work will indirectly be used in different fields such as in varietal selection, plant ideotype designing and in genetic studies.

Because of a lack of time, we couldn't analyze yet the data collected on NPT and IR72 plants. This will be done subsequently. Even if these plants were not grown until maturity, the data collected until flowering will be sufficient to predict the sink size. The experiment comparing two densities of population of IR72 plants will help characterizing the effect of changes in plant density on the overall framework.

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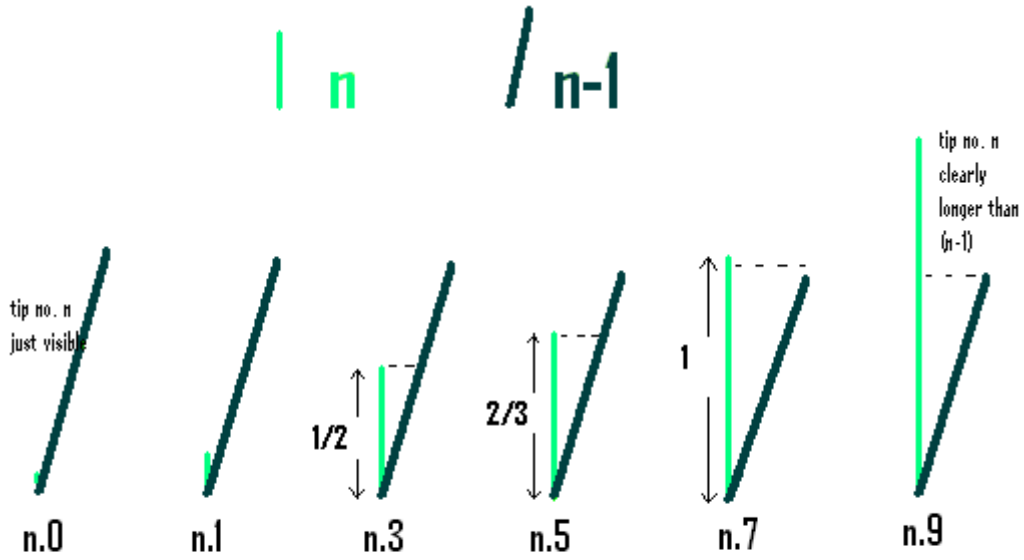
ANNEX

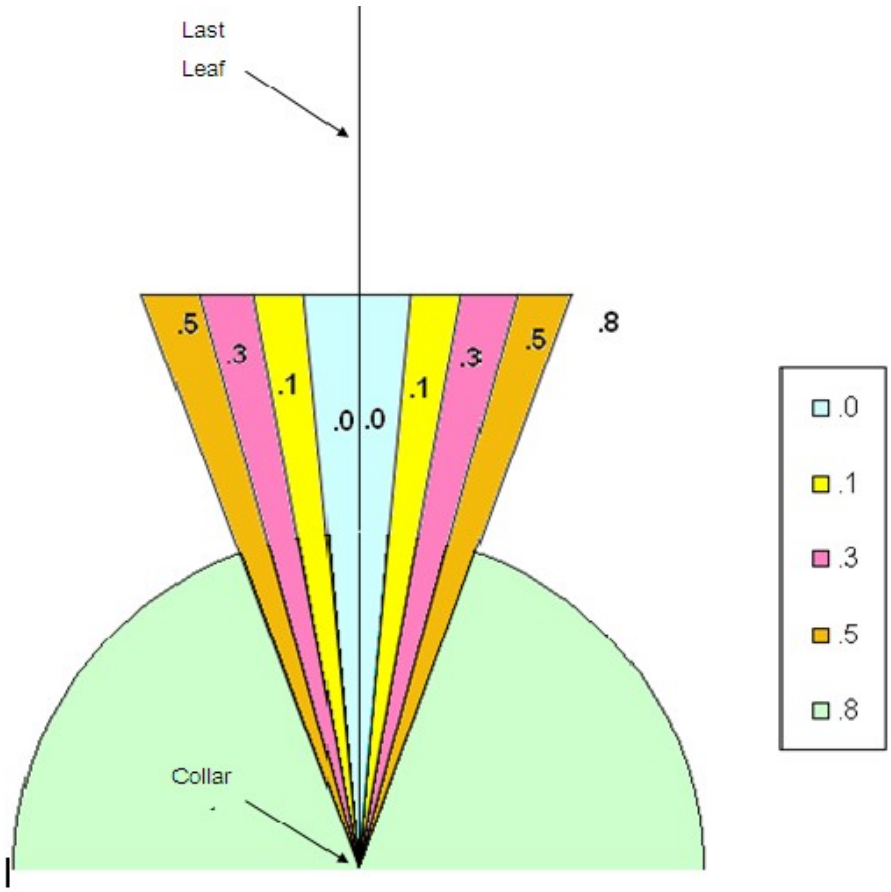
GROWTH PHASE	STAGE	DAS (Day After Sowing)
Vegetative phase	Beginning of Tillering	18-21
	Mid-Tillering	32-36
	Panicle Initiation / Max-Tillering	42-48
Reproductive phase	Booting	2 weeks after PI (56-62)
	Flowering (~50% of the panicle flowered)	73-78
Ripening phase	Mid Grain Filling	2 weeks after Flowering (87-92)
	Maturity	105-108

Annex 1: Stages in rice plant growth



Annex 2: Decimalization of the number of visible leaves.

The last emerged leaf is in light green, the next to last emerged leaf is in dark green. For instance, if the leaf number 10 has just emerged, we count 10,0 visible leaves. If the leaf number 10 is much longer than the leaf number 9, we consider that its growth in length is almost finished and leaf number 11 is about to emerge: we count 10,9 visible leaves.



Annex 3: Decimalization of the number of collars

Decimalization is done using the angle between the last two emerged leaves.