Analysis of the variability

of cardinal temperatures in rice

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Key words: Cardinal temperatures, Leaf Elongation Rate, *Oryza Sativa*, tropical *japonica*, phenotyping

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Content

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Jayani Perera

Abstract: Rice is one of a world's most important food crops. Rice is exclusively grown in all over the world, in various agro ecosystems. Climatic changes, loss of agricultural lands because of urbanization are some of the critical points in rice production in the world. In this experiment we phenotyped 198 tropical *japonica* rice accessions for their cardinal temperature (T_{base},T_{opt} and T_{max}) with the objective of studying their diversity. Precise cardinal temperatures and knowledge on the diversity is very important in breeding programs and crop improvement. We used a method based on leaf elongation rate (LER) to determine the cardinal temperatures. Leaf elongation rates were determined at six different temperatures; 16, 20, 23, 26, 30 and 35°C. The relationship between LER and temperature followed a beta distribution curve with cardinal temperatures as parameters.

Results obtained with high precision presenting a good diversity for the three cardinal temperatures among the *japonica* accessions we used. T_{base} range was between around 8°C and 16°C. The range of optimum temperature was between around 28 °C and 38 °C. The maximum temperature range was between 34 and 55°C. These cardinal temperatures were significant with the varietal type (traditional and improved). The base temperatures were significant with the genetic groups defined by a previous microsatellite study.

Main imperfection of this method is that it is a time consuming task. Broaden the temperature ranges at the two extremes will facilitate to have more precise values for T_{base} and T_{max}. Determine the cardinal temperatures of *indica* and temperate *japonicas* will permit to have a better knowledge of the diversity of the all *Oryza* groups.

Key words: Cardinal temperatures, Leaf Elongation Rate, *Oryza Sativa*, tropical *japonica*, phenotyping

Résumé : Le riz est la culture alimentaire la plus importante dans le monde. Les différents écosystèmes de production du riz se retrouvent sur l'ensemble de la planète. Les phénomènes de changement climatique, la diminution des superficies rizicultivées pour des raisons d'urbanisation sont les principaux frein a la production rizicole actuelle. Dans ce travail nous avons phénotypé les températures cardinales (T_{base}, T_{opt} et T_{max}) pour 198 variétés japonica tropicaux L'obtention de valeurs precises et l'étude de la diversité sont particulièrement importante pour les programmes de sélection et l'amélioration de la culture.

Nous avons utilisé une méthode basée sur la vitesse d'élongation des feuilles (LER) pour déterminer les températures cardinales. Les vitesses d'élongations ont été déterminées à 6 températures différentes (16, 20, 23, 26, 30 et 35 °C). La relation entre LER et température suit une distribution de type beta ayant comme paramètre les températures cardinales.

Les résultats ont été obtenus avec une bonne précision et présentaient une grande diversité pour le panel de variété japonica tropicaux utilisé. T_{base} variait de 8 à 16 °C, T_{opt} se situait entre 28 et 38 °C et T_{max} se modulait entre 34 et 55 °C. Ces valeurs de températures cardinales étaient corrélées avec le type de variété (traditionnel ou amélioré). Plus spécifiquement, seul Tbase a montré une corrélation avec les groupes génétiques obtenus par microsatellites lors d'une étude précédente sur ce même panel.

La principale limite d'utilisation de cette méthode est le temps nécessaire a sa réalisation. La précison des valeurs de T_{base} et T_{max} peut etre amélioré en augmentant le spoints de mesures aux températures extremes. La connaissance destemperatures cardinales des *japonicas* tempérés et des *indica* permettrait de mieux connaitre la diversité présente sur l'ensemble de l'espèce *Oryza.*

1. Introduction

1.1. General context

Climatic changes are one of the major problems in rice cultivation. Using a number of scenarios, the Intergovernmental Panel on Climate Change (IPCC) estimates that between 1990 and 2100, temperature is likely to rise by 1.4 to 5.8°C. Although the likely effect of this rise in temperature is regionally dependant, it is thought that in most tropical and subtropical areas (where tropical rice is grown), crop yields are likely to decrease with increasing temperature, exacerbated by a reduction in rainfall (Parent *et al.*, 2010).

Rice is the main food crop in the world. With the accrescence of world population and abatement of the arable land, food problem may become more severe in next few years. Therefore some strategies are required to ensure food security. One way is to increase rice productivity through varietal improvement. For that, to get a better knowledge on physiological mechanism of adaptation to the constraints induced by climate change is needed.

In non water limiting conditions, temperature is the most important parameter that interacts with crop growth and productivity. There are three most important temperature points in plant growth: basal temperature, optimum temperature and maximum temperature. They are called cardinal temperatures. Although there are a lot of parameters available to measure plant growth, we propose to use leaf elongation rate as the basis to determine the cardinal temperatures of a large collection of rice accessions composed mostly of tropical japonicas .

We then intend to link these data with the genetic diversity to study how these phenotypic variations associate with genetic diversity and how this trait is genetically controlled. The genetic diversity of the japonica rice varieties has been analyzed by using previously obtained SSR data. An association mapping study between the phenotypic diversity and genetic diversity of these varieties using SNP data enabling to identify markers linked to loci controlling the trait will be later undertaken, but is not a part of this work.

A better understanding on the mechanism and the genetic diversity of the traits in rice will help for crop improvement through plant breeding with association mapping and marker based selections.

We propose to phenotype the cardinal temperatures of a panel of 198 varieties. This panel represents the genetic diversity of the tropical Japonica sub species with accessions collected from various countries.

1.2. Rice (*Oryza sativa*)

Rice is consumed as a grain almost exclusively by humans, supplying 20% of daily calories for the world population (World Rice Statistics, http://www.irri.org; FAOSTAT, http://apps.fao.org). Rice is a member of Poaceae family. Rice shows wide adaptations to climate and cropping systems. It has a large genetic and morphological diversity too (about 100 000 accessions in IRRI gene bank).

Oryza sativa, a diploid species with n =12 chromosomes, has two sub species, *japonica* and *indica*. In ecological terms, *indica* accessions are primarily known as lowland rice that is grown throughout tropical Asia. They are irrigated varieties. The sub species japonica can be found in two forms; tropical japonica and temperate japonica. The tropical japonica accessions are grown without irrigation, (rain-fed upland rice) while temperate japonica accessions are irrigated.

As a model organism with a fully sequenced genome, rice affords unique opportunities to use genomic approaches to study its domestication, adaptive diversity, and the history of the crop improvement. *O.sativa* is predominantly autogamous and therefore gene flow is restricted. As a result geographically or ecologically distinct groups of rice are expected to show greater genetic differentiation than would be an out crossing species (Garris *et al.,* 2005).

The rice plant usually takes 3-6 months from germination to maturity. This duration depends on the variety and other climatic conditions. During this period, rice complete two distinct sequential growth stages namely vegetative and reproductive. The vegetative stage refers to a period from germination to the initiation of panicle primordia, the reproductive stage from the panicle initiation to heading and the ripening period from the heading to maturity.

The vegetative stage is characterized by active tillering, gradual increase in plant height, and leaf emergence at regular intervals. Tillering may start when the main culm develops the 5^{th} or 6^{th} leaf. The reproductive growth stage is characterized by culm elongation, decline in tiller number, and emergence of the flag leaf, booting, heading and flowering.

A typical rice leaf is composed of the sheath, the blade, the ligule and the auricle. The sheath is an elongated, ribbon shaped leaf base rolled into a cylinder that encloses all the younger parts of the shoot. The blade is narrow, flat and longer than the sheath in all leaves except the second. The ligule is a small, white, triangular scale. A pair of hairy and sickle shaped auricles are located at the junction between the collar and the sheath.

At a given time, the rice plant is composed of leaves that are physiologically different in age and activity. The rate of leaf emergence is affected by temperature.

1.3. The ORYTAGE project

The objective of the ORYTAGE project is to develop an international phenotyping network for rice adaptations to drought and thermal stresses in the context of gene discovery and association mapping. In this project three panels of rice varieties were chosen which belong to tropical *japonica*, temperate *japonica*, and *indica* sub species. One activity of the project is to phenotype all these varieties for their response to a large number of stresses. The phenotypes of the tested lines will be compared with their molecular polymorphism using a 1 million SNP chip. Temperature is one of the stresses that have been considered. Cardinal temperatures play an important role for adaptation to temperature stress

1.4. Importance of Temperature in rice growth

Temperature is the single most important factor regulating germination of non-dormant seeds in annual agro ecosystems at the beginning of the growth season where light, nutrients and moisture are typically not growth limiting (Garcia-Huidobro *et al*., 1982). It is an important factor which determines the yield too. All biological processes respond to temperature, and all responses can be summarized in terms of three cardinal temperatures, namely the base or minimum (T_{min}), the optimum (T_{opt}), and maximum (T_{max}) temperatures (Yan and Hunt., 1999). The minimum or basal temperature is the lowest temperature at which a plant is able to grow. The optimum temperature is a temperature at which the highest rate of growth is obtained in a given period of time and the maximum temperature is the highest temperature in which a plant can grow.

The combination of temperature and time is a major appropriate unit of measure for predicting plant development than time alone (Ritchie and NeSmith, 1991). Various forms of temperature summations, commonly referred to as thermal units or growing degree days, have been utilized in numerous studies to predict phenological events for both agronomic and horticultural crops (Baker and Reddy, 2001).

Leaf growth is controlled by a complex network of factors (Poire et al., 2010). Some of these factors are endogenous regulatory mechanisms that determine leaf shape (Wyrzykowska et al., 2002; Rolland-Lagan et al., 2003), the progression of the cell cycle (Tsukaya and Beemster, 2006), and the relationship of leaf growth to the circadian clock (Nozue and Maloof, 2006). Other factors can be regarded as external, such as the recurring changes of day and night, alterations in temperature, or further physical, chemical, or biotic parameters, which can increase or decrease growth at various time-scales (Granier and Tardieu, 2009; Walter et al., 2009).

Temperature changes induce immediate and clear responses of growth, but it is still uncertain to what extent this is mediated via signaling cascades (Penfield, 2008; Franklin, 2009)

In monocotyledonous species, leaf elongation rate largely follows temperature alterations (Ben-Haj-Salah and Tardieu, 1995; Pietruszka et al., 2007). Cell division rate may have a role in the control of leaf expansion rate (Salah and Tardieu., 1995). Temperature has a major effect on leaf expansion rate because cell division rate can be greatly affected by temperature (Francis and Barlow., 1988), and partly explains both day/night alternations and longer-term changes in elongation rate of monocot leaves (Watts, 1974; Gallagher and Biscoe, 1979; Kemp and Blacklow, 1980).

Although it originated in the tropics, rice is cultivated widely from tropical through temperate areas. The effect of temperature on rice production is very divergent and complex. It affects production directly or indirectly, for example, through outbreak of diseases or changes in soil conditions. The effect of temperature differs among different physiological properties and different organs of the rice plant. There are some other factors such as developmental age, Varity, cultivation methods, and environmental conditions.

Cardinal temperatures are important data for plant improvement. Rice varieties which are have low basal temperatures, have better cell activity in a long part of the day. It means that in those plants, physiological processes such as photosynthesis and enzyme reactions are going on well. Rice varieties with low basal temperature are important for cultivation in high altitudes where very low temperatures are encountered. A low basal temperature reduces the time gap between development stages and it is important to determine the sowing date. Rice phenology is directly affected by cardinal temperatures, so determination of cardinal temperatures help to predict the best timing for crop management intervention. A low basal temperature is also important for high yield because low temperature at panicle initiation leads to spikelet sterility, thereby to yield losses. Optimum temperature is important to have a maximum vegetative and reproductive growth rate.

1.5. Association mapping

The objective of genetic mapping is to identify simply inherited markers in close proximity to genetic factors affecting quantitative traits.

Rice genetic mapping often involves the development, genotyping and phenotyping of double haploid, recombinant inbred or advanced back cross lines derived from an F1 cross between different cultivars (Agrama et al, 2007).

The central problem with the above approach is the limited number of meiosis that have occurred in the mapping population and the cost of propagate lines to allow for a sufficient number of meioses. In addition, it takes a considerable time too. As an alternative for this, association mapping has been introduced. Compared to traditional linkage analysis, association mapping using collection of varieties offers three advantages.

1. Increased mapping resolution because of a linkage disequilibrium lower in a collection of varieties
2. Immediate availability of the population and therefore, reduced research time
3. Greater allele number.

Because of these advantages, association mapping is now more and more widely using in plant genome analysis. Both linkage analysis and association studies rely on polymorphism of molecular markers. These molecular markers should have a high degree of polymorphism, should cover the genome at a high density and preferably co-dominance inheritance.

Another drawback of association mapping is the need to take population structure into account to avoid high rates of false positive in the association analyses

Use of SSR markers to interpret population structure results in much greater resolution than use of other types of markers, because of the high level of polymorphism of SSR (Akkaya et al, 1992). It provides a great tool in study of population genetics, when associating this high level of polymorphism

with the low level of homoplasy observed in *O. sativa*. Although SSR is a good marker, it may not reach a density sufficient for association studies. Therefore SNP, which occurs at a density of 1 SNP per 100 bp in rice, is the most frequently used molecular marker in association studies.

1.6. Objectives

Overall objective of the work

Determine the cardinal temperatures for each variety through leaf elongation measurement.

Specific objectives

Compare the phenotypic variability for cardinal temperatures with the genetic diversity of the panel in view of association studies.

2. Material and method

2.1. Materials

In this experiment, 173 japonica rice varieties and 25 reference varieties belonging to other varietal groups were used to determine their cardinal temperatures. For the japonicas, rice varieties which were used in this experiment originated from 32 different countries, and represented the diversity of the sub species. They included both traditional varieties and improved varieties. They are listed in Appendix 1. They have been characterized with 25 microsatellite markers that enabled to determine the putative number of sub populations in the collection.

2.2. Methods

To determine cardinal temperatures, we can use either growth or development of plants. In this experiment we used plant growth, using LER as the key-parameter. Although a lot of parameters are available to determine cardinal temperatures, such as plant dry weight, chlorophyll content or rate of photosynthesis, we decided to use LER, as it is compatible with the time of the study and the facilities available. Especially, here we used 198 japonica rice varieties and LER is the better and easier parameter to use in determining cardinal temperatures. Leaf elongation is directly related with plant growth and development and it represents a lot of physiological processes. So it is directly related with yield too.

2.2.1. The principle

Leaf elongation is not a single process. It is a result of a group of mechanisms which are controlled by external and internal factors although it is not well known to which extent temperature is one of a major factor which controls these mechanisms.

In this experiment we used the beta distribution model developed by Yan and Hunt, 1999. This model can be used to determine the temperature response in several plant processes. The model is important because of several reasons;

1. It has only three parameters namely, maximum temperature, optimum temperature and maximum growth rate.
2. All three parameters are self-explanatory and have clear biological definitions.
3. It gives a smooth curve rather than several lines.
4. It deals with the plant response to the whole range of temperatures, rather than just a fraction of them.

- Step 1: Determining of the Leaf Elongation Rate (LER)

Leaf elongation of rice follows the normal growth curve of plants (Figure 1). It consists of three main phases: lag phase, exponential phase and continuous phase. By using this curve, leaf elongation rate can be determined through a logistic model (equation 1). This logistic model gives the LER at inflection point and it is the maximum LER at the particular temperature.

$$f(t) = K\frac{1}{1+a\,e^{-rt}} \qquad \text{(Equation 1)}$$

This slope or LER therefore analytically can be expressed as below. It is a derived form of equation 1.

$$\text{LER} = \frac{Kr}{4} \qquad \text{(Equation 2)}$$

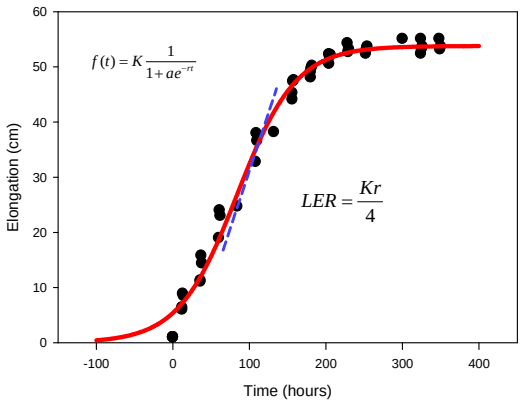

Figure 1: curve of the leaf elongation

- Step 2: Determining of cardinal temperatures.

After determination of leaf elongation rates (LER) at particular temperatures, the graph of temperature Vs LER is drawn. As discussed by Shaykewich (1995), the typical first leg of a biological temperature response curve is of a generalized logistic type (Yan and Hunt, 1995, equation 3). The biological response or activity increases slowly at around the base temperature and above and then it increases linearly with the temperature. Then it increases slowly and reaches to a maximum level at the optimum temperature. After the optimal temperature, the activity decreases and ceases at just below the maximum temperature (Figure 2).

Here the LER gives as a function of temperature.

$$LER = Rmax \left(\frac{\max(0,Tmax-t)}{Tmax-Topt}\right)\left(\frac{\max(o,t-Tmin)}{Topt-Tmin}\right)^{\frac{Topt-Tmin}{Tmax-Topt}} \quad \text{(Equation 3)}$$

Cardinal temperatures could be directly determined by using this model. The optimal temperature is determined with the maximum of the curve and the maximum temperature is determined with the curve intersection with temperature axis.

Base temperature can be determined by using three methods. They are:

1. By extending the graph until it intersect the X axis.
2. By using extended line, which drown across the slope of the inflection point of the curve
3. Remove all the points after optimal temperature and extend the linear region of the graph until X axis

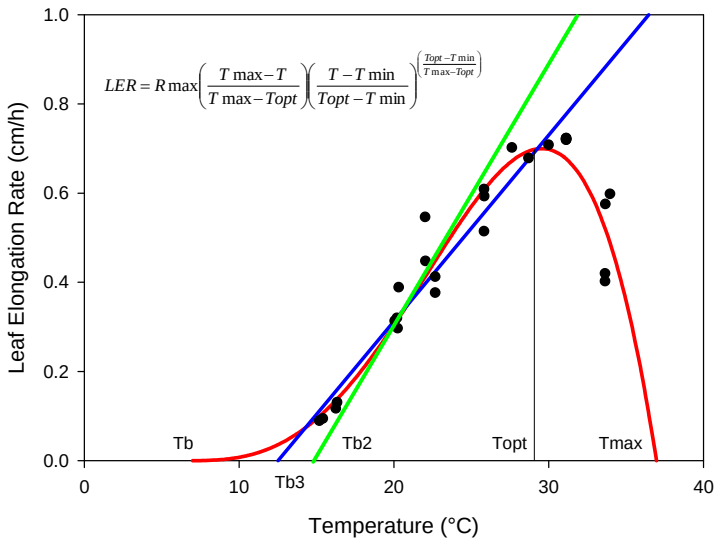

Figure 2: The biological temperature response curve to determine Cardinal temperatures (Tb, T_{opt} and T_{max}) and two methods of determining the base temperature (Tb1 and Tb2).

2.2.2. Procedure

We planned the experiment to minimize the time waste between experiments. We prepared new sets of plants while one experiment was going on. Leaf elongation measurements were performed at six different temperatures (16°C, 20°C, 23°C, 26°C, 30°C and 34°C).

- Germination

Four seeds were selected from each variety. Seeds which were more golden and plump were selected. Selected seeds were free of dirt and not opened. Seed germination was performed in small boxes. The boxes were cleaned and dried well. A small piece of blotting paper was placed at the bottom of each box. Then the seeds were placed on the blotting paper, separated from each other and wet with distilled water. They were incubated at 30°C for 2 days.

- Transplanting

Seedlings were planted in pre-prepared pots. One liter volume pots were filled with the medium which was a mixture of compost Nehaus-S and pozzolana (5.7%). First, a half of the pot was filled with the medium. Then 2g of fertilizer was added into it and mixed well with the medium. The pot was filled with the medium again and packed well. A gap of one inch was left between the edge of the pot and medium level. Medium in the pot was soaked with water.

Two good and nearly same size seedlings were selected from each variety and planted in the same pot. After the seedlings were well established, at the 2 leave stage, the best seedling was selected among the two.

Plants were grown in a growth chamber under controlled conditions, until the appearance of the fifth leaf. The average temperature inside the growth chamber was 25 °C day and 26 °C night with a photoperiod of 12 hours. The conditions in the growth chamber were managed to obtain a good plant growth. The plants were well watered, with good fertilization and 70% of relative humidity. The temperature, light intensity and relative humidity were recorded with a datalogger.

- Transferring to the phytotron/ cold chamber

When the fifth leaf appeared, the plants were transferred to the phytotron. The positions of the plants on the table were changed every day to minimize the effect of the possible heterogeneity of light intensity and temperature. Measurements of leaf growth were not made on the fifth leaf but on the sixth. Thus the shift into growth chamber at this stage allows the plant to adapt to the temperature to which it is submitted, before measurements. Because of limited technical means, two growth chambers were used: a cold growth chamber at 16°C and the other for warmer temperatures (20°C, 23°C, 26°C, 30°C and 34°C). These conditions were controlled by a datalogger as in the growth chamber.

- Measurement of leaf growth

The leaf elongation was measured every day, approximately at the same time of the day to limit bias. Measurements were made on the sixth leaf. The rice plant takes 2-3 months from germination to maturity, depending on the variety and the existing environmental conditions. Normally tillering begins

to occur from the 4th leaf stage and stops at panicle initiation. When the rice plant is in its 6th leaf stage, it reaches the maximum growth rate. We began to measure the leaves just after the 6th leaf appeared. Measurements were made daily between the ligule of the fifth leaf and the tip of the sixth leaf until it became a constant value, by using a vertically mounted ruler. The ruler has been clamped vertically to facilitate to obtain the exact value.

- Links between phenotypic and genetic diversity

To explain the phenotypic diversity, we used passport data and genetic data available for the population. Passport data included country of origin, region of origin, varietal type (traditional or improved). Genetic data included varietal group (tropical or temperate) and genetic groups defined by the software “Structure” based on SSR data. We performed an analysis of variance on Tbase-reg, T opt and T max using passport or genetic information as source of variations.

3. Results

3.1. Leaf Elongation Rates and Cardinal Temperatures

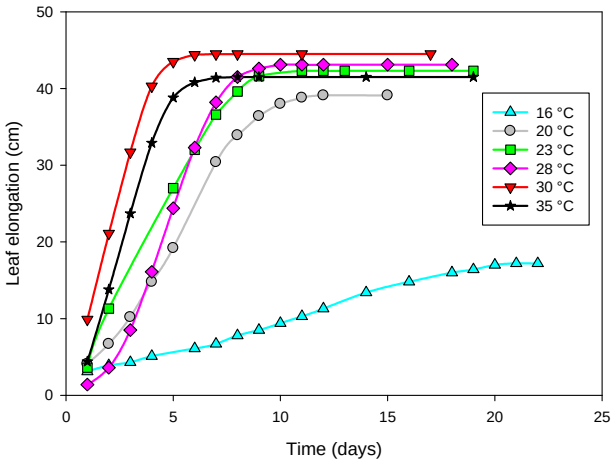

Figure 3: Differences of leaf elongations of variety APO at the six different temperatures

We observed very clearly that there was a temperature effect on rice leaf elongation. As shown in Figure 3, there was a small leaf elongation at 16^0C and it increases with the temperature. But at 35 ^{0}C the leaf elongation has reduced. At 16^0C, the length of the leaf is very short and the length was getting longer with the temperature. But at 35^0C, again the leaf is becoming shorter compared to lengths at other temperatures.

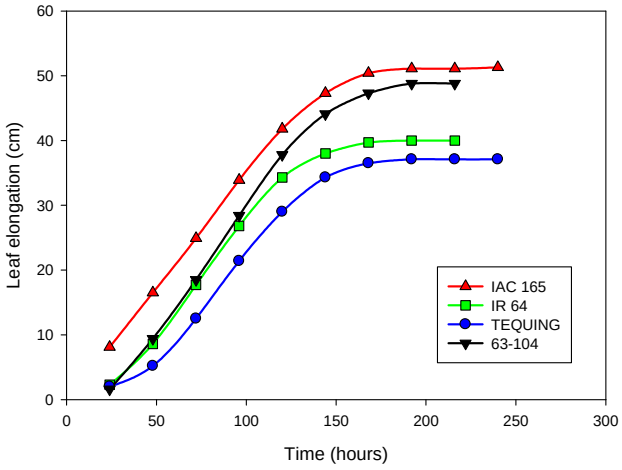

Figure 4: Varietal effect on leaf elongation at 26°C in four varieties

We observed also a varietal effect on leaf elongation. As shown in Figure 4, the leaf elongation was not same between the varieties at the same temperature. Some varieties had a lower leaf elongation rate and some had higher leaf elongation rate.

With the leaf elongation data we obtained, we determined leaf elongation rates by using the model (step 1, equation 1). The model is very well calibrated with the leaf elongation data as it is shown in Figure 5. With this good adjustment, we could determine LERs with very low slandered errors by using the equation 2 (figure 5).

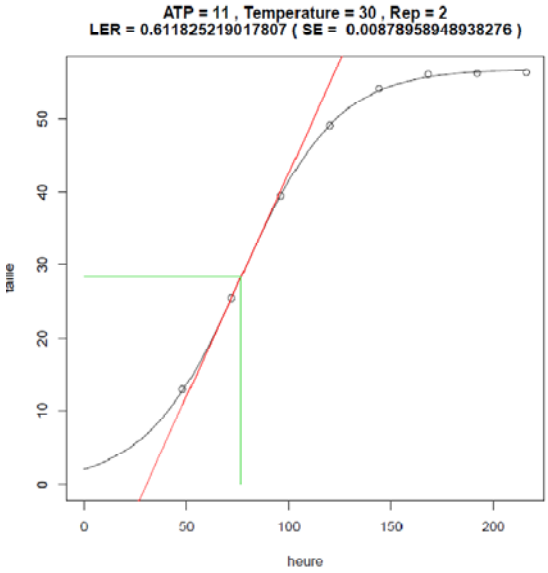

Figure 5: Leaf elongation curve of the rice variety FR13A (ATP 11) at 30 ^{0}C

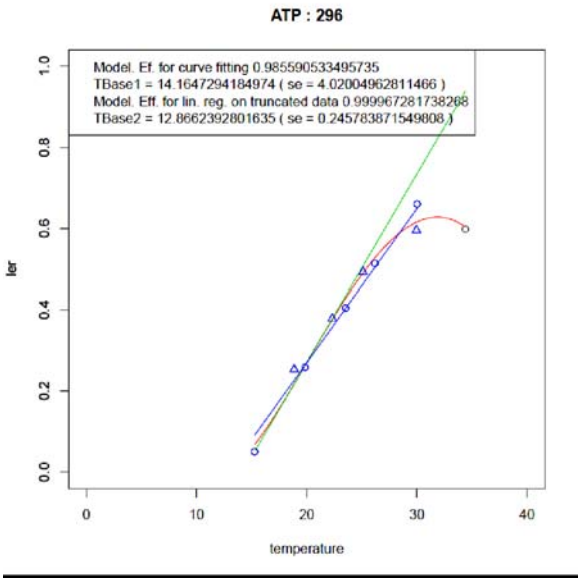

Figure 6: Temperature function of the leaf elongation rates of the rice variety DINORADO (ATP 296)

We saw that mean leaf elongation rates increased with temperature but at 35°C it begun to decrease. The mean standard error and standard deviation were also very low. Table 1 gives an overall idea about the varieties that were including in the panel. According to these results we can say that the overall experiment was going well and the measurements were well fitted with the model.

Table 1: Summary of descriptive statistics for LER values of 198 japonica rice varieties.

Temperature °C	observed mean temperature	LER min	LER max	Mean LER	LER SD	Mean SE
16	15.972	0.020	0.421	0.073	0.033	0.004
20	19.263	0.115	0.422	0.225	0.045	0.060
23	22.639	0.227	0.515	0.355	0.059	0.011
26	25.656	0.226	0.965	0.480	0.099	0.017
30	29.611	0.255	0.811	0.555	0.078	0.019
35	34.245	0.105	0.840	0.504	0.111	0.023

With the model we obtained cardinal temperatures (step 2, equation 3). The results of last year trial and the results of this year trial overlapped well (Figure 6). The step 2 model is also well adjusted to the data. However the cardinal temperature obtain present variable precision, we obtained some unlikely values for T_{base}, when we extend the curve to find the T base (Annex 1). But using the other two methods, we obtained better values for T_{base} which were compatible with the literature data. Using regression method we obtained more realistic values compared to other 2 methods (Table 5). We observed the same problem, when we determine the T_{max} using this model.

Table 2: Statistics of cardinal temperatures

	No of accessions	mean	mean SE	min	max
R max	190	0.575	0.041	0.36	0.83
Tbase(By model)	165	12.52	14.39	2.46	17.97
Topt	185	30.96	1.49	27.72	37.70
Tmax	177	41.33	4.26	34.82	59.47
Tbase-tangent	188	14.86	1.39	12.92	17.92
Tbase regression	188	13.07	1.26	8.51	15.92

In this experiment, we could determine the three cardinal temperatures (T_{base}, T_{opt} and T_{max})for 183 varieties correctly. Another 9 varieties were doubtful, as the points were not well fitted with the model we used. There were another 6 varieties for which we could not determine the optimum temperature and the maximum temperature with the temperature range that we used in this experiment.

3.2. Diversity of the cardinal temperatures

We observed a good diversity among these accessions of japonica varieties for three cardinal temperatures (figure 5). The temperature range for T_{base} was between 8 and 16 °C. The temperature range for T_{opt} was between 27 and 36 ^{0}C. Temperature range for T_{max} was between 35 and 53 ^{0}C. Generally, most of the varieties had a T_{base} around 13-14 ^{0}C, T max around 38-42 ^{0}C and T_{opt} around 28-31^{0}C.

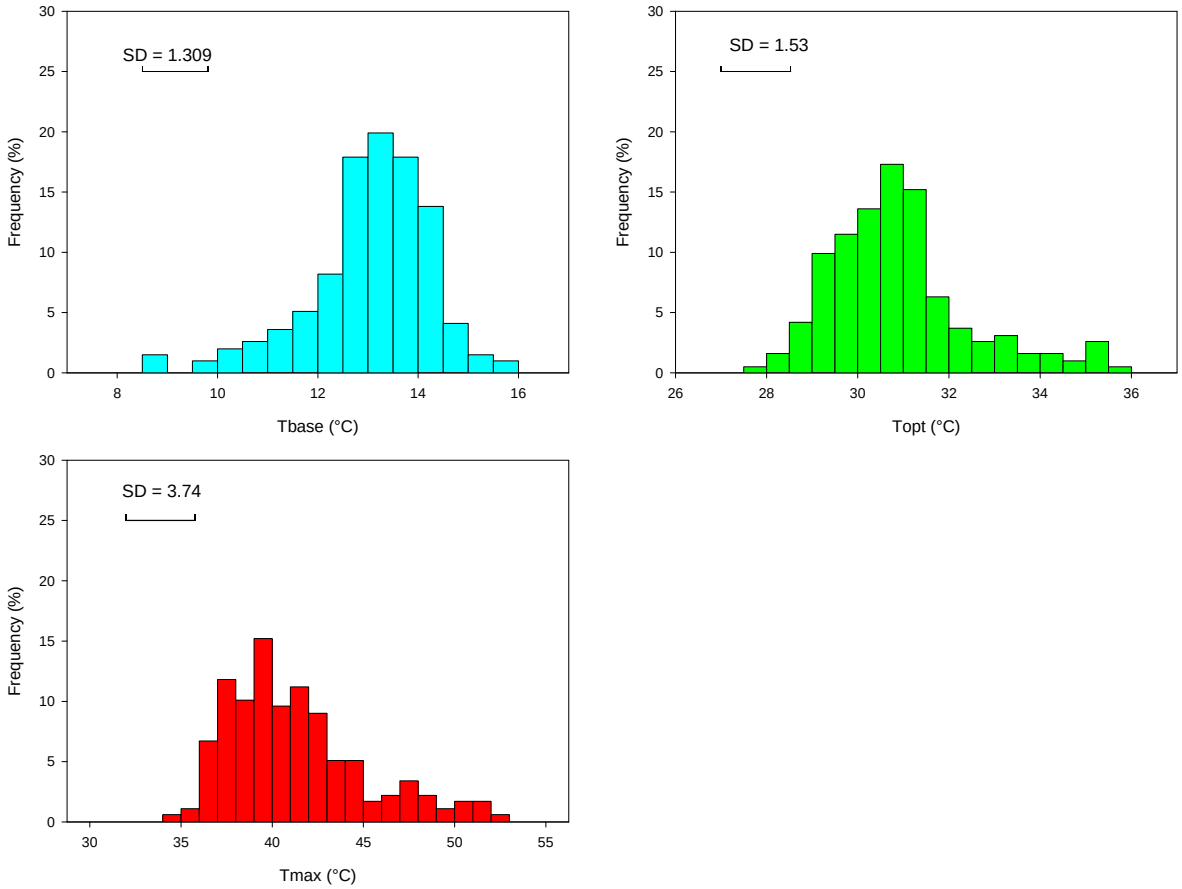

Figure 5: Histograms of T_{opt}, T_{max} and T_{base} values. Histogram 1 shows the range of basal temperatures (obtained by using method 3). Histogram 2 shows the range of optimum temperatures and histogram 3 shows the rage of maximum temperatures of this rice panel. All the histograms follow normal distribution.

3.3. Study of correlation between different groups and cardinal temperatures

Then we tried to explain the factors causing differences between varieties. We grouped the varieties used, into several categories with both passport and genetic data (Annex 1). Then we tried to find a correlation between cardinal temperatures and these data by performing a variance analysis. We saw that there was no correlation between the country of origin of the accessions and cardinal temperatures. But we found that there was a correlation between, the agro-ecological region and T_{opt}. All three cardinal temperatures were significant with the varietal type (Table 3 and Table 5).

With the previously obtained SSR data, we tried to make some genetic groups by using the software "Structure". It was very difficult to define the exact number of sub populations (K). Therefore we tesed the K value up to K=7. But we removed K=3, K=5 as they were very unlikely. The ANOVA was significant for T base at both K=2 and K=4 (Table3, Table 6 and Table 7).

Table 3: Results of the variance analysis conducted on japonica accessions

		Topt			Tmax			TBaseReg		
	groups	df	F	P	Df	F	P	df	F	P
Passport	Country	29	1.48	0.0701	29	0.97	0.522	31	1	0.4706
Passport	Region	**10**	**2.4**	**0.0095**	10	1.78	0.0682	10	1.77	0.0695
Passport	Type	**1**	**11.69**	**0.0008**	**1**	**6.94**	**0.0093**	**1**	**6.37**	**0.0125**
Genetic structure	VG	1	2.74	0.0996	1	0.75	0.387	1	0.92	0.3401
Genetic structure	K2	1	1.12	0.2922	1	4.37	0.0388	**1**	**12.03**	**0.0007**
Genetic structure	K4	3	2.67	0.0518	3	1.63	0.1877	**3**	**4.82**	**0.0035**

Table 4: Mean comparison between T opt in different regions

SNK grouping		Mean	N	Region
	A	31.8063	9	West and Central Africa_T
B	A	31.4277	11	South Asia_T
B	A	31.3987	35	Equatorial Asia_T
B	A	31.2502	11	Latin America_T
B	A	31.1028	15	Madagascar_T
B	A	30.5974	3	East Asia_T
B	A	30.4958	18	Equatorial Asia_I
B	A	30.463	19	West and Central Africa_I
B	A	30.2787	26	Latin America_I
B	A	30.1655	15	South East Asia_T
B		29.1582	3	Temperate_I

The mean T_{opt} create two clear separate groups between varieties collected from west and central Africa and varieties collected from temperate regions. The mean T opt values is higher in the varieties which has been collected from African region. All the other groups do not have a considerable differences between their T opt values.

Table 5: Mean comparison between cardinal temperatures in different types.

	SNK grouping	Mean	N	Type
T opt	A	31.1665	99	traditional
	B	30.66	99	improved
T max	A	42.7797	100	traditional
	B	40.5598	66	improved
T base	A	13.2805	69	Improved
	B	12.7902	103	traditional

Table 6: Mean comparison between Tbase in different genetic groups (K=2)

SNK grouping	Mean	N	K2
A	13.4073	62	2
B	12.9462	45	3 (admixed)
B	12.6141	65	1

Table 7: Mean comparison between T base in different genetic groups (K=4)

SNK grouping		Mean	N	K4
	A	13.5354	42	2
B	A	13.1452	24	4
B	A	12.8489	62	5 (admixed)
B	A	12.7447	26	1
B		12.3209	18	3

4. Discussion

This trial is the second replication of a large experiment. Last year, the same experiment has been performed with the same conditions. This year we have done the second replication minimizing differences between conditions of two experiments as much as possible. We were working with a panel which consists of a large number of *japonica* varieties. With the available facilities, we were not able to grow two replicates per variety at a time. The phytotron have a capacity to carry 200 plants at a time. Therefore we decided to split the experiment along time. It is not sufficient to use only one plant per variety to have good results with minimum standard error. If we have a problem with one variety we can check it with the second trial. No big problem occurred during the experiment. Seed germination and growth were normal.

Globally, we obtained good results except for a few varieties were the cardinal temperatures were not realistic and with a high standard error. For theses varieties, (6) the model could not be adjusted to the extreme temperatures (low and high). With the available facilities and capacities, we were able to determine the leaf elongation rate only between 16^0C and 35^0C temperature range. For a better

adjustment, the LER at temperatures below 16^0C and above 35^0C are necessary. To determine the cardinal temperatures of these 6 varieties, we will have to broaden the temperature range. In these cases, we were just able to determine the base temperature by using linear regression method.

There were another few varieties, which the results are doubtful as the points we obtained from the experiment are not well fitted with the model. These points could be explained by experimental errors, bad growth and development, diseases etc.

The model appears well adapted to determine the cardinal temperatures. We could obtain good results for 183 varieties (92.4%) out of 198. It was quite easy to determine the T_{opt} with this model, because T_{opt} is the maximum value of the curve. But we had some problems with determining T_{base} and T_{max} using this model. As mentioned above, we did not have facilities enabling to test temperatures below 16^0C and above 35^0C. Because of we do not have data below 16^0C we do not know exactly what happen below this temperature. Therefore when we extend the curve to determine the basal temperature (method 1), there was a great possibility to have unlikely under estimations. In the opposite, the method 3 (use the tangent at the inflexion point) showed over estimated values (13-17°C). Using linear regression method (method 3) we could have more reasonable values related to the literature (around 11-13°C).

The same problem occur when determine the T_{max} too. As we do not have data above 35 ^{0}C we cannot predict precisely how plants behave at high temperatures. Sometimes the growth can stop suddenly instead of decreasing gradually as explained in the model. We don't know the biological reality of $T_{max.}$

To increase the accuracy of our results we can do several things. One suggestion is to have more replicates. Once we have the values for cardinal temperatures we can use the exact temperature range with small intervals and redo the experiment to have more precise values for each cardinal temperature. But this is a time consuming and fastidious task, notably at low temperatures. The results of these two experiments were quite good. If we do a third replication, it may enable us to estimate cardinal temperatures for the varieties that were having problems.

According to our knowledge this is the first time that a large number of *japonica* accessions have been phenotyped at once. It is a great advantage to have phenotypic data for a panel consisting of a large number of accessions for genetic studies and crop modeling.

By using our results we tried to classify the accessions in our panel according to the range of temperature in which they can be cultivated successfully (Figure 8). Some scientists predict that the varieties which are well adapted to high temperatures are not well adapted to cold temperatures. But according to our results there is a large diversity of these ranges. Some plants can only be cultivated in a very narrow temperature range but some plants can be grown in a very wide range of temperatures.

For T_{base}, we got even around 8^0C for some varieties. And also for T_{maxt} we got some results more than 50^0C (figure 5). In biological means, these ranges of temperatures may not be suitable for many

of biological processes such as germination, tillering, grain filling or flowering. Rice pollen is known to be killed at 43^0C. Both lower and higher temperatures may cause spikelet sterility, hence reduce the yield. But as we used leaf elongation as our parameter, these extreme temperatures may not be as much a problem for leaf elongation

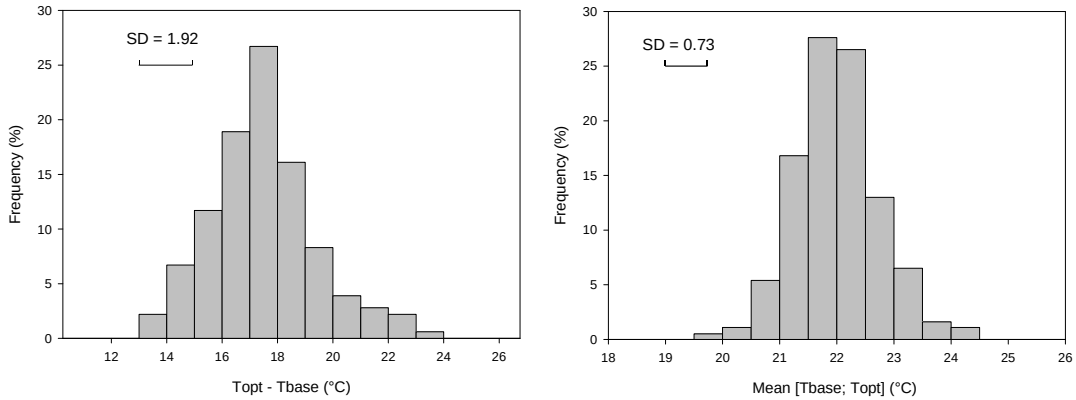

Figure 8: Range of different temperature windows

According to some literature data, leaf elongation has relatively low basal temperature and high maximum temperature (table 8). Anyway we observed very slow growth rates at both lower and higher temperatures. In addition to that we observed chlorotic areas on the leaves at lower and higher temperatures. We suppose that this phenomenon occurred due to poor activity of enzymes. *Japonica* rice varieties are more tolerant to low temperatures than *indica* varieties. For example, critical low temperature for photosynthesis has been reported as 18.5°C for japonica rice and between 20 and 25°C for *indica* rice (Yoshida *et al*, 1976).

Table 8: Response of the rice plant to varying temperature at different growth stages (Yoshida *et al*, 1981)

	cardinal temperatures ^{0}C		
growth stage	T_{base}	T_{opt}	T_{max}
germination	10	20-35	45
seedling emergence and establishment	12-13	25-30	35
Rooting	16	25-38	35
Leaf elongation	7-12	31	45
Tillering	9-16	25-31	33
Initiation of panical primordia	15	-	-
panical differentiation	15-20	-	38
Anthesis	22	30-33	35
Ripening	12-18	20-25	30

We could not obtain any correlation between cardinal temperatures and countries, because even within a country there can be various climatic zones. For an example even Sri Lanka is a small island, there are seven agro-climatic zones. So if we consider the whole country it may be quite difficult to find a relationship. Then we grouped the varieties according to agro climatic regions. We

found a relationship between T_{opt} and agro climatic regions. If consider the mean comparison, we can see two distinctly separated groups. One group consists of the accessions collected from West and Central Africa and the other group consists of the accessions collected from temperate regions. The accessions from the West and Central Africa show a higher mean T_{opt} value compare to the mean T_{opt} value of the accessions from Temperate regions showing that these accessions are better adapted to the climatic conditions which they naturally belong to. In the temperate regions irrigated rice cultivars starts when spring temperatures are between 13^0C and 20^0C and the crop is harvested before temperature drop below 13^0C in the autumn (Yoshida, 1981). But in tropical countries, the average temperature is high all over the year.

We obtained a good correlation between varietal type and cardinal temperatures. We divided the rice varieties into two groups (traditional or improved varieties). These traditional varieties have been collected from different areas in the world. And these traditional varieties are normally adapted to the climatic conditions of the environment to which they naturally belong. But an improved variety is a result of breeding program involving a series of hybridizations. The parents may come from distinct areas. The cardinal temperatures of the improved varieties are less extreme than those of the traditional groups. It's likely that the breeding process was associated with some degree of loss in adaptation.

Then we associated these data with varietal groups. We categorized all japonica varieties into two groups, a temperate and a tropical. There was no significant correlation between the varietal group and cardinal temperatures. The panel we used mostly included tropical japonica rice varieties. There were only a few varieties which represented temperate accessions. So it may be the reason for this kind of results.

When further analyze these genetic groups we saw that at K=2, there was two separate groups, one consisting of varieties coming from Asian countries and the other group consisting of Africa, Latin America and Improved varieties. The mean T_{base} values of these two groups show a significant difference. At k=4, we saw another 3 groups which were not easy to characterize while the group of Asian rice varieties remaining as same. The fact that there is not a very strong relationship between the panel structure and the cardinal temperatures should facilitate association analysis.

We would like to propose to do the same experiment with *indica* rice varieties and temperate *japonica* rice varieties. It is very important to have a general view of the diversity of cardinal temperatures of these sub species for crop improvement

5. Conclusion

Determination of cardinal temperatures is a long process. Although it could be able to determine the cardinal temperatures easily by using the beta distribution model, it takes a long time to produce data (grow plants in each temperature) however the method used, is well adapted to determine the cardinal temperatures. By using the beta distribution model, we could determine precisely the cardinal temperatures of 198 japonica rice varieties. For this panel of rice varieties, T_{base} values lie between 8.51°C and 15.92°C. T_{opt} values lie between 27.72°C and 37.70°C. T_{max} values lie between 34.82°C

and 59.47°C. Two replications are enough to determine cardinal temperatures, but a third trial would be an added advantage for few varieties which showed a bad fitting. A good diversity for the three cardinal temperatures is exists among these japonica accessions. This diversity is correlated with thevarietal type (traditional or improved) the knowledge of cardinal temperatures may be interesting for plant breeding programs and crop improvements.

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ATP	Name	Country	Type
1	APO	PHILIPPINES	I
2	ASD 1	INDIA	T
4	AZUCENA	PHILIPPINES	T
6	BASMATI 370	INDIA	T
7	BULU PANDAK	INDONESIA	T
8	DOM SOFID	IRAN	T
9	DULAR	INDIA	T
10	FANDRAPOTSY 104	MADAGASCAR	T
11	FR13A	INDIA	T
12	GAMBIAKA	BURKINA FASSO	T
13	GIZA 171	EGYPT	I
14	IAC 165	BRAZIL	I
15	IR64	PHILIPPINES	I
16	KAUKKYI ANI	MYANMAR	T
17	KHAO DAM	THAILAND	T
18	KHAO DAWK MALI 105	THAILAND	T
19	M 202	USA	T
21	MOROBEREKAN	GUINEA	T
22	N 22	INDIA	T
23	NIPPONBARE	JAPAN	I
25	TEQUING	CHINA	T
250	62667	SENEGAL	T
251	63-104	SENEGAL	T
252	ARAGUAIA	BRAZIL	I
253	ARIAS	INDONESIA	T
254	ARROZ CEBADA	VENEZUELA	T
255	BABER	INDIA	T

Annex 1: Details of the japonica varieties used in this experiment

256	BAGANAN ASALAO	MALAYSIA	T
257	BAKUNG H	MALAYSIA	T
258	BELOHAKILA 119	MADAGASCAR	T
259	BENGALY VAKARINA	MADAGASCAR	T
260	BICO BRANCO	BRAZIL	T
261	BINULAWAN	PHILIPPINES	T
262	BODA 148-3	MADAGASCAR	T
263	BOTRA FOTSY	MADAGASCAR	T
265	CAAWA/FORTUNA 6	PHILIPPINES	I
266	CAIAPO	BRAZIL	I
267	CANA ROXA	BRAZIL	T
268	CANELA DE FERRO	BRAZIL	T
269	CHA LOY OE	THAILAND	T
270	CHA PHU MA	THAILAND	T
273	CICIH BETON	INDONESIA	T
274	CIRAD 141	BRAZIL	I
275	CIRAD 358	IVORY COAST	I
276	CIRAD 392	MADAGASCAR	I
277	CIRAD 394	MADAGASCAR	I
278	CIRAD 402	BRAZIL	I
279	CIRAD 403	Unknown	I
280	CIRAD 409	Unknown	I
281	CIRAD 488	MADAGASCAR	I
282	CNA-7\BO\1\1>33-13-6-1	COLOMBIA	I
283	COLOMBIA 1	COLOMBIA	I
284	CT13582-15-5-M	COLOMBIA	I
289	CUBA 65	CUBA	T
290	CUIABANA	BRAZIL	I
291	CURINCA	BRAZIL	I
292	DAM	THAILAND	T
293	DANGREY	BHUTAN	T
294	DAVAO	PHILIPPINES	T
295	DAWASAN RED	BHUTAN	T
296	DINORADO	PHILIPPINES	T
297	DOURADO AGULHA	BRAZIL	T
298	DOURADO PRECOCE	BRAZIL	T
299	EH IA CHU	TAIWAN	T
300	ESPERANZA	BOLIVIA	T
301	FOHISOMOTRA	MADAGASCAR	T
302	FOSSA HV	BURKINA FASSO	T
303	GANIGI	INDONESIA	T

304	GEMJYA JYANAM	BHUTAN	T
305	GOGO	INDONESIA	T
306	GOGO LEMPUK	INDONESIA	T
307	GOGO LEMPAK	INDONESIA	T
308	GOMPA 2	INDIA	T
310	GRAZI	IVORY COAST	T
311	GUARANI	BRAZIL	I
312	GUNDIL KUNING	INDONESIA	T
314	HAWM OM	THAILAND	T
315	HD 1-4	FRANCE	I
319	IAC 25	BRAZIL	I
320	IAC 47	BRAZIL	I
321	IDSA 77	IVORY COAST	I
322	IGUAPE CATETO	BRAZIL	T
323	INDANE	MYANMAR	T
325	IR60080-46A	PHILIPPINES	I
326	IR63371-38	PHILIPPINES	I
327	IR63380-16	PHILIPPINES	I
328	IR63372-08	PHILIPPINES	I
330	IR65907-116-1-B	PHILIPPINES	I
331	IR65907-188-1-B	PHILIPPINES	I
332	IR66421-096-2-1-1	PHILIPPINES	I
334	IR68704-145-1-1-B	PHILIPPINES	I
335	IR70758-17-2-1	PHILIPPINES	I
336	IR71525-19-1-1	PHILIPPINES	I
337	IR71676-90-2-2	PHILIPPINES	I
338	IR72967-12-2-3	PHILIPPINES	I
339	IRAT 104	IVORY COAST	I
340	IRAT 109	IVORY COAST	I
341	IRAT 112	IVORY COAST	I
342	IRAT 13	IVORY COAST	I
343	IRAT 144	GHANA	I
344	IRAT 170	IVORY COAST	I
345	IRAT 177	FRENCH GUYANA	I
346	IRAT 2	SENEGAL	T
347	IRAT 212	IVORY COAST	I
348	IRAT 216	IVORY COAST	I
349	IRAT 234	GUF-BRA	I
350	IRAT 257	BRAZIL	I
351	IRAT 335	BOLIVIA	I
352	IRAT 362	NICARAGUA	I

353	IRAT 364	NICARAGUA	I
354	IRAT 366	NICARAGUA	I
355	IRAT 380	MADAGASCAR	I
356	JAO HAW	THAILAND	T
357	JIMBRUK JOLOWORO	INDONESIA	T
358	JUMALI	NEPAL	T
359	JUMULA 2	NEPAL	T
360	KAKANI 2	NEPAL	T
361	KANIRANGA	INDONESIA	T
362	KARASUKARA SURANKASU	TAIWAN	T
363	KEDAYAN	MALAYSIA	T
365	KENDINGA 5 H	MALAYSIA	T
367	KETAN KONIR	INDONESIA	T
368	KETAN LUMBU	INDONESIA	T
369	KETAN MENAH	INDONESIA	T
371	KHAO KAP XANG	LAOS	T
372	KINANDANG PATONG	PHILIPPINES	T
373	KOMOJAMANITRA	MADAGASCAR	T
374	KU 115	THAILAND	I
375	KUROKA	JAPAN	T
377	LAMBAYQUE 1	PERU	T
378	LOHAMBITRO 3670	MADAGASCAR	T
379	LUDAN	PHILIPPINES	T
380	MA HAE	THAILAND	T
381	MAINTIMOLOTSY 1226	MADAGASCAR	T
382	MALAGKIT PIRURUTONG	PHILIPPINES	T
383	MANANELATRA 520	MADAGASCAR	T
384	MANDRIRAVINA 3512	MADAGASCAR	T
385	MARAVILHA	BRAZIL	I
387	MITSANGANAHIJERY	MADAGASCAR	T
388	MOLOK	INDONESIA	T
389	NABESHI	TAIWAN	T
390	NEP HOA VANG	VIETNAM	T
391	NHTA 10	INDIA	T
392	NHTA 5	INDIA	T
393	NPE 253	PAKISTAN	T
395	NPE 826	PAKISTAN	T
397	ORYZICA SABANA 6	COLOMBIA	T
398	OS 4	ZAIRE	T
399	OS 6	ZAIRE	T
400	P5589-1-1-3-P	COLOMBIA	I

401	PACHOLINHA	BRAZIL	T
403	PADI BOENAR	INDONESIA	T
404	PADI KASALLE	INDONESIA	T
407	PALAWAN	PHILIPPINES	T
410	PCT11\0\0\2,BO\1>55-1-3-1	COLOMBIA	I
412	PCT4\SA\4\1>1076-2-4-1-5	COLOMBIA	I
413	PEH PI NUO	CHINA	T
414	POENOET HITAM	INDONESIA	T
416	PULU LAPA	INDONESIA	T
417	RATHAL	SRI LANKA	T
418	REKET MAUN	INDONESIA	T
419	RT 1031-69	ZAIRE	T
420	SA TANG	LAOS	T
421	SEBOTA 65	BRAZIL	I
422	SENG	THAILAND	T
424	SPEAKER	PHILIPPINES	T
427	TANDUI	MALAYSIA	T
428	TREMBESE	INDONESIA	T
429	TRES MESES	BRAZIL	T
430	TSIPALA 89	MADAGASCAR	T
432	VARY LAVA 90	MADAGASCAR	T
433	VARY LAVA DE BETAFO	MADAGASCAR	T
434	VARY MADINIKA 3566	MADAGASCAR	T
435	VARY MALADY	MADAGASCAR	T
436	VARY MANANELATRA	MADAGASCAR	T
437	VARY SOMOTRA SIHANAKA	MADAGASCAR	T
438	WAB 56-125	IVORY COAST	I
439	WAB 56-50	IVORY COAST	I
440	WAB706-3-4-K4-KB-1	IVORY COAST	I
442	Y CHANG JU	CHINA	T
443	YANCAOUSSA	IVORY COAST	T
444	YANGKUM RED	BHUTAN	T
445	YUNLU 7	CHINA	T
446	IR47686-09-01-B-1	PHILIPPINES	I
447	IR53236-275-1	PHILIPPINES	I
448	IR65261-09-1-B	PHILIPPINES	I
449	IR65261-19-1-B	PHILIPPINES	I
451	IR65907-173-1-B	PHILIPPINES	I
452	IR65907-206-4-B	PHILIPPINES	I
453	IR66421-105-1-1	PHILIPPINES	I
454	IR71524-44-1-1	PHILIPPINES	I

455	VIETNAM1	VIETNAM	T
456	VIETNAM2	VIETNAM	T
457	VIETNAM3	VIETNAM	T
458	IR47684-05-1-B	PHILIPPINES	I
459	PRIMAVERA	BRAZIL	I
999	EARLY MUTANT IAC 165	BRAZIL	T