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Rice Breeding for High-yield, Cold Tolerance and Blast Disease Resistance through the Utilization of Unexploited Genetic Resources

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Yunnan province, China, is believed to be one of the areas where rice cultivation originated. This provided a suitable background for starting a collaborative research program on rice breeding, based on the exchanges of genetic materials from both countries, following a formal accord signed by Agriculture Ministers of two governments. Since 1982, the Tropical Agriculture Research Center, Japan and the Yunnan Academy of Agricultural Sciences, Peoples' Republic of China, have been implementing a joint research program entitled "Breeding of Rice Varieties for High-Yield and Resistances to Cold Weather and Blast Disease through the Utilization of Unexploited Genetic Resorces". The main objectives of the program are as follows :

(1) Breeding of japonica rice varieties adapted to the Yunnan conditions.
(2) Development of breeding methods for the effective utilization of unexploited genetic resources.
(3) Exchange and utilization of genetic resources of rice based on a principle of reciprocity and equality.

Key words : new japonica rice varieties, cross-hybridization,

Materials and Methods

(1) Japanese rice variety "Todoroki-wase" was chosen as a parent material for cross-hybridization.
(2) Chinese rice varieties "Yunjing No.135" and "Yunjing No.9" were also used as parent materials for cross-hybridization.
(3) In 1983, cross-hybridization between the Japanese variety and Chinese varieties was initiated.
(4) The rapid generation advance method was used for the F_1 and F_2 progenies derived from cross-hybridization at Hainan island.
(5) After the F_3 progeny, the selection for high-yield and resistances to cold weather and blast disease was initiated within the Yunnan district and 13 lines were eventually selected from the progeny.
(6) 13 lines of "Hexi No.1-No.13" were tested for their performance including yield, resistance to cold weather and blast disease and some other characteristics, in more than 12 different locations in the Yunnan district.
(7) 4 lines of "Hexi No.2, 4, 5 and 10" out of 13 lines showed good performance in the above tests during a period of 2 years. These 4 lines were examined for their adaptability in the large scale verification trials conducted in farmers' fields.

Dian Jing No.18

Results and Discussion

(1) In 1990, the provincial government of Yunnan officially approved the registration of three new cultivars developed through the Sino-Japanese cross-hybridization programs, recognizing the superior performances demonstrated by these cultivars in the province. The area planted with these cultivars exceeded 20,000 ha in 1990. Their characteristics are described as follows :

1. Dian Jing No.18 = Hexi No.4 : This progeny from the cross Todoroki-wase x Yunjing No.135, is characterized by a high-yield, early maturity, intermediate plant type, cold resistance and blast resistance.
2. Dian jing No.19 = Hexi No.5 : This progeny from the cross Todoroki-wase x Yunjing No.135, is characterized by an intermediate plant type, high-quality of hulled rice, blast resistance and intermediate maturity.
3. Dian jing No.20 = Hexi No.10 : This progeny from the cross Todoroki-wase x Yunjing No.9, is characterized by an intermediate plant type, blast resistance, high-yield and intermediate maturity.

Dian Jing No.20

(2) The comparison for cold tolerance of Japanese breeding materials with the Chinese ones revealed that the Yunnan materials contained some cultivars which were highly tolerant to cold weather.

(3) Application and subjects for further research : The planting area to the varieties developed which covered more than 20,000 ha in 1990 will be increased in future.

The cooperative research program between TARC and the Chinese institutes should be continued as it is important to breed rice varieties for higher resistance to cold weather and blast disease, and for higher-quality of hulled rice and resistance to other major diseases and insect pests.

Table 1.
Major characteristics of the three new varieties.

Name of variety Promising lines	Dian Jing No.18 Hexi No.4	Dian Jing No.19 Hexi No.5	Dian Jing No.20 Hexi No.10	Yun Jing No.9
Maturity	Early	Intermediate	Intermediate	Late
Plant type denoting Yield dependence	Intermediate	Intermediate	Partial panicle weight	Panicle weight
Heading/Ripening time	Jul.16/Aug.31	Jul.26/Sep.5	Jul.23/Sep.12	Jul.30/Sep.16
Culm height cm	83	84	86	101
Panicle height cm	17.2	17.0	15.7	16.0
Number of panicles/m^2	714	438	509	418
Color of apiculus	Brown	White yellow	White yellow	White yellow
Shattering habit	Limited	Intermediate	Intermediate	Very limited
Lodging resistance	High	High	High	Low
Leaf blast resistance	High	High	High	Intermediate
Panicle blast resistance	Very high	Very high	Very high	High
Genotype for blast resistance	Pi-i	Pi-i	+	+
Tolerance to cold weather	Very high	Intermediate	High	Very high
Yield (unhulled) kg/a	97.9	79.9	98.6	82.7
Yield (hulled) kg/a	80.3	68.9	79.7	69.4
1,000 graing weight g	22.8	22.6	19.1	19.7
Quality of grain	Intermediate	Good	Intermediate	Poor

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Proposal of a Method for Differentiating Races of *Pyricularia oryzae* Cavara in the *Japonica* Rice Cultivated Area of Yunnan Province, China

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The blast caused by *Pyricularia oryzae* Cav. is most destructive disease of rice in Yunnan Province, China (Fig. 1). Growing resistant varieties is the most effective and economical countermeasure to protect the crop from the disease. The mechanisms of blast resistance are classified into two categories, i. e., "true resistance" and "field resistance". The true resistance is characterized by its specificity to each race of the blast fugus and occasionally called vertical resistance, while the field resistance is non-specific, being called horizontal resistance.

Since breakdowns of the highly resistant varieties dependent on true resistance genes have occurred frequently in several countries including China, breeding programs in most countries aim to develop a high level of field resistance rather than the true resistance.

In order to establish an effective breeding program for durable resistance to the blast disease, it is necessary to look in more details into the pathogenic specialization of the blast fungus. This research was conducted to select a set of differentials from commercial *japonica* rice varieties in Yunnan Province.

Key words ; rice, blast, resistance, physiological race

Classification of Japonica rice varieties based on reaction patterns to blast fungus isolates

Two hundred twenty-six rice varieties bred in twelve agricultural science institutes in Yunnan Province, twenty-nine Japanese varieties which were already known for genotypes for true resistance, and nine Japanese differential varieties were tested in the experiments. The varieties were inoculated with six isolates collected in Yunnan Province as shown in Table 1 and classified into varietal groups on the basis of their specific reaction patterns.

Two hundred twenty-six *japonica* rice varieties in Yunnan Province were divided into seven groups, i. e., from I to VII. Varieties of Group I, IV and II, III were further divided into three and two subgroups, respectively (Table 1).

Research Period : 1987-89
Research Site : Yunnan Academy of Agricultural Sciences, Peoples' Republic of China

Fig 1.
Paddy field with rice plants showing blast disease
A : Rice plants infected with blast fungus
B : Rice plants showing a high resistance as they carry a resistance gene against blast fungus

Table 1.
Classification of *japonica* rice varieties in Yunnan Province based on reaction patterns to Yunnan blast fungns isolates

Group	Sub-group	Y-34 (001)	Y87-018 (003)	Y88-15 (007)	Y88-14 (017t+)	Y88-25 (037t+)	Y88-45 (037t+)	Number of varieties
I	1	+	+	+	+	+	+	48
	2	+	+	+	+	−	+	1
	3	+	+	+	−	+	+	1
II	1	−	+	+	+	+	+	31
	2	−	+	+	−	+	+	1
III	1	−	−	+	+	+	+	40
	2	−	−	+	+	−	−	4
IV	1	−	−	−	+	+	+	34
	2	−	−	−	+	−	+	4
	3	−	−	−	+	−	−	5
V	1	−	−	−	−	+	+	24
VI	1	−	−	−	−	−	+	5
VII		−	−	−	−	−	−	28

+ : Susceptible − : Resistant,
The figures in parentheses indicate the Japanese race number of the fungus isolates

Table 2.
Classification of the varieties of Group VII based on reaction patterns to eight isolates with different pathogenicities

Group	Sub-group	Y88-436 (103b+)	Y88-24 (303b+)	Y-69 (137b+)	HA89-41 (403)	Y-73 (433)	TH81-04 (437)	HA89-23 (303)	TH77-1 (047)
VII	1	+	+	+	−	−	−	−	−
	2	−	−	+	−	−	−	−	−
	3	−	−	−	+	+	+	−	−
	4	−	−	−	−	−	−	−	−

+ : Susceptible − : Resistatnt
Y : Isolate from Yunnan Province HA : Isolate from Hainan Island
TH : Isolate from Japan
The figures in parentheses indicate the Japanese race number of the fungus isolates

Group I - 1 corresponds to shin 2[1)] type in terms of the reaction pattern to blast races and each one variety of Group I - 2 and I - 3 might have a resistance gene or genes not to be indentified yet. Group II - 1 corresponds to Aichiasahi type[1)] and the varieties in this group have the gene *Pi-a*. Group III - 1 corresponds to Ishikarishiroke type[1)] or Shinsetsu type[1)] and the varieties in this group have the gene *Pi-a* or *Pi-a* and *Pi-i*. Group IV - 1 corresponds to Kanto 51 type[1)] or To-to type[1)], and this group may include varieties of the following three different genotypes, i. e., *Pi-k*, *Pi-a Pi-k* and *Pi-i Pi-k*, Group V - 1 also corresponds to Kanto 51 or To-to type, and this group may include varieties of the following different genotypes, i. e., $Pi\text{-}k^m$, $Pi\text{-}a\ Pi\text{-}k^m$ and $Pi\text{-}i\ Pi\text{-}k^m$. Group VI - 1 corresponds to Yashiromochi type[1)] and the varieties in this group have the gene *Pi-ta*.

Twenty eight varieties of the Group VII were resistant to all the isolates inoculated. To classify them further into subgroups, the varieties of the Group VII were inoculated with eight isolates which were virulent to the varieties having at least one of the following genes, i. e., *Pi-z*, $Pi\text{-}ta^2$, $Pi\text{-}z^t$ and *Pi-b*. Varieties of the Group VII were divided into four subgroups as shown in Table 2. Group VII - 1 and VII - 2 may have the gene *Pi-b*, *Pi-b* and/or other gene or genes, respectively. Varieties of the Group VII - 3 may have the gene $Pi\text{-}z^t$.

Selection of differential varieties

Several varieties which formed many susceptible--type lesions or showed highly resistant reaction were selected from Group I - 1, II - 1, III - 1, IV - 1, V - 1, VI - 1, VII - 1, VII - 3 and VII - 4, respectively. Those varieties were inoculated with 86 isolates collected in Yunnan Province, and a total of nine varieties as shown in Table 3, each of which may have a single discrete resistance gene and showed stable reaction to the isolates were selected as exemplified by the differential varieties from each group. As there was not suitable variety, Tsuyuake that was adopted as the representative of Group Ⅴ-1.

Nomenclature of the race

Gilmour's octonal notation according to the proposal of Yamada *et al.* 's proposal[2] was adopted for differentiating races. First, 9 differential varieties are arranged in the order listed in Table 3, and a code number was given to each variety. The number of each race is the sum of the cord numbers. For example, the race virulent to Lijiang-xintuanheigu, Yunjin 20, 04-2685 and Hexi 16 is 1+2+40+200=243. Y denotes Yunnan Province.

This method for differentiating races will be contribute much to Programs for breeding varieties resistant to blast disease in Yunnan Province.

Table 3.
Reaction of some isolates on the differential varieties

Differential varieties	Presumed genotype	Code number	Isolate				
			Y-34	Y87-18	Y88-15	Y88-45	Y-65
Lijian-xintuanheigu	+	1	+	+	+	+	+
Yunjin 20	*Pi-a*	2	−	+	+	+	+
7907-1	*Pi-i*	4	−	−	+	+	−
Kunming-beizigu	*Pi-k*	10	−	−	−	+	−
Tsuyuake	*Pi-k*^{m}	20	−	−	−	+	−
04-2685	*Pi-ta*	40	−	−	−	+	+
82-12	*Pi-z*^{t}	100	−	−	−	−	−
Hexi 16	*Pi-b*	200	−	−	−	−	+
Ziyu 44	Unknown	400	−	−	−	−	−
Race			Y1	Y3	Y7	Y77	Y243

+ : Susceptible
− : Resistant

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Research Period : 1985-89
Research Site : ICRISAT
(International Crops Reserch Institute for the Semi-Arid Tropics)

Special Mechanism of Phosphorus Uptake by Pigeonpea and its Role in Cropping Systems of the Indian Subcontinent

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Phosphorus (P) is normally the most limiting nutrient for the growth of leguminous crops in tropical and subtropical regions. In Alfisols, one of the major soils in the semi-arid tropics, the content of iron or aluminum oxide is high and P is strongly bound and largely unavailable for crop uptake. Pigeonpea (*Cajanus cajan* (L.) Millsp.), a legume crop widely cultivated as an intercrop with cereals and other crop species in the semi-arid regions, is generally observed to yield better than other crops in low-P soils even without P fertilizer application. In this study, the mechanisms underlying the efficient P uptake by pigeonpea were explored and comparisons made with other crop species. These mechanisms are discussed in relation to the improvement of the P fertility of soils in low-input cropping systems of the Indian sub-continent.

Key words: *Cajanus cajan*, phosphorus, piscidic acid, iron-bound phosphorus, root exudates

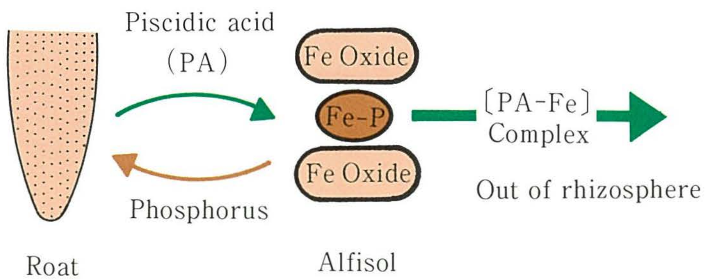


Fig 1.
Postulated mechanism, for the absorption of iron-bound phosphorus from soil by pigeonpea.

Soil	Pigeonpea	Soybean	Sorghum	Pearl millet	Maize
Alfisol	5.72	1.40*	0.59*	0.64*	0.51*
Vertisol	2.34	6.53	3.91	5.38	6.13

Table 1.
P contents (milligrams of P per pot) of shoot of crop plants at the grain-filling stage. Plants were grown in potted alfisol or vertisol in the greenhouse, without P addition.
* Plants died 1 month after sowing.

Materials and methods

Pigeonpea (cultivar ICPL 87), sorghum (cultivar CSH 5) and several other crops were used as plant materials. Among the alfisols and vertisols at the ICRISAT Center (18°N, 78°E), a representative sample of each soil with low P availability was selected (4.1 and 0.7 mg P/kg soil, respectively, Olsen's extraction method). Pot experiments were conducted in a greenhouse. Field experiments were conducted at the Center under rain-fed conditions with supplementary irrigation. Root exudates were collected from 2-months-old plants grown on sand. The ability of the fractions of root exudates to solubilize $FePO_4$ was tested by the addition of 20 ml of the fraction to a test tube containing 10 mg of $FePO_4$. After shaking for 30 min, the P content was determined in the supernatant.

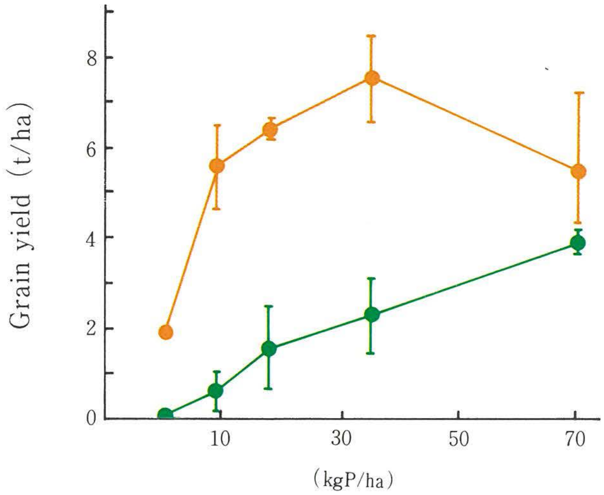
Results and Discussion

1. When no phosphorus was applied, the crops grew well on the vertisol, but not on the alfisol due to phosphorus deficiency except for pigeonpea (Table 1). In the alfisol, most of the P is associated with iron (Fe-P) which is unavailable to most of the crops. The results obtained suggest that the better growth of pigeonpea on alfisols was related to the unique ability of the crop to utilize Fe-P, the dominant form of P in alfisols.
2. The analysis of root exudates showed that the ability to solubilize Fe-P in pigeonpea root exudates was associated with the presence of piscidic acid, a phenolic compound, which is absent in the root exudates of other crops such as soybean and sorghum. The proposed mechanism is that piscidic acid forms chelate compounds with the Fe^{3+} component of Fe-P resulting in the release of P (Fig 1).
3. On an Alfisol where the available phosphorus level was very low, sorghum yield was nearly nil without P fertilizer, but it was much improved and a yield of 2 t/ha was achieved after the cultivation of pigeonpea (Fig 2).

These results indicate that the roots of pigeonpea solubil-

Fig 3.
Intercropping of pigeonpea with sorghum

Fig 2.
Effect of previous pigeonpea on P response of sorghum in an alfisol field of low P fertility
• First year sorghum
• Sorghum after pigeonpea



Phosphorus applied

ized Fe-P in soil and made it available to the following crop.

Application and future research needs

1. The results indicated that the introduction of pigeonpea into the cropping system increased the level of available phosphorus in soil.
2. Due to the ability to utilize P from Fe-P, pigeonpea does not unduly compete with companion crops for fertilizer P or other sources of available P such as Ca-P. Thus the intercropping of pigeonpea and other companion crops such as sorghum, which has been traditionally practiced in the Indian sub-continent, appears to be an efficient system for the utilization of the limited amount of phosphorus in soil (Fig 3). This mechanism of intercropping may be utilized for further development of such a cropping system.
3. In view of the increasing cost and scarcity of soluble P fertilizers, especially for resource-poor farmers in marginal environments, the identification of pigeonpea genotypes or other crop species with high efficiency in the use of relatively insoluble P sources would be important.

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Genesis and Distribution Patterns of Red-Yellow and Related Soils in the Philippines

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The objectives of the study were ; to analyze the genesis, physico-chemical and mineralogical properties of Red-Yellow and related soils in the Philippines ; to determine their patterns of distribution in relation to parent materials, topographic position and climate ; to classify these soils according to some soil classification systems ; and to evaluate their productivity potential in implementing agricultural development programs in the Philippines.
Key words : soil genesis, patterns of soil distribution, Red-Yellow soils, ultisols, the Philippines

Materials and Methods

Nine areas all over the Philippines were selected and soil samples from 48 pedons were collected and analyzed. The primary considerations for selecting the sites and the pedons were as follows :

1) Red-Yellow soils and related soils in the Philippines which were temporarily classified into 13 soil taxa depending on the kind of parent materials and topography ; and
2) Climate in the Philippines which was grouped into 3 types based on the duration of dry months ; namely : 5 dry months or more (tropical monsoon climate), 2-4 dry months (tropical monsoon climate), and less than 2 dry months (tropical rain forest climate).

Results and Discussion

1. Main upland soils in the Philippines consisted of Red soils, Yellow soils, Dark Brown soils, Terra・rossa-like soils, Terra・fusca-like soils, Rendzina-like soils, Brown Upland soils, Pseudogley-like soils, Andsols, Vertisols, Brown Lowland soils, and Gray Lowland soils.

2. From residual, old alluvial and volcanic mud flow, andesite and basalt materials, Red soils and Yellow soils were formed on hills, and upper and middle terraces.

The Red soils were strongly acid with a low activity under climatic conditions characterized by less than 2 dry months, strongly to moderately acid with a low activity under climatic conditions characterized by 2 to 4 dry months, and moderately acid under climatic conditions characterized by more than 4 dry months. The Yellow

Research Period : 1986-90
Research Site : College of Agriculture, University of the Philippines, Philippines

Profile of Kandic-pseudogleyed Red soil (Kandiudult)

Profile of Kandic Yellow soil (Kandihumult) soils were strongly acid with a low activity under climatic conditions characterized by less than 2 dry months, strongly to moderately acid under climatic conditions characterized by 2 to 4 dry months, and were hardly formed under climatic conditions characterized by more than 4 dry months.

On lower terraces, only Yellow soils were formed. The Yellow soils were moderately acid with a low activity under climatic conditions characterized by less than 2 dry months, and moderately acid under climatic conditions characterlized by 2 or more dry months.

These Red-Yellow soils were classified as Kandihumults, Kandiudults, Kanhapludults, Haplohumults or probably Haplustults in the soil taxonomy.

3. From residual, aquatic and/or eolian materials on limestone, Red soils were formed on hills, Terra・rossa-like soils on flat higher terraces, Terra・fusca-like soils on flat lower terraces, and Rendzina-like soils on recently raised coral terraces and slope of rolling terraces. Red soils were strongly to moderately acid, Terra・rossa-like soils slightly acid, Terra・fusca-like soils slightly acid to neutral, and Rendzina-like soils neutral to slightly alkaline.

Terra・rossa-like soils and Terra・fusca-like soils were classified as Paleudalfs, Hapludalfs, Paleustalfs or Haplustalfs, and Rendzina-like soils as Rendolls or Haplustolls in the soil taxonomy.

4. Under climatic conditions characterized by more than 4 dry months, Brown Upland soils with soft powdery lime (Ustropepts) developed from residual material of calcareous shale and sandstone on hills, and Vertisols with soft powdery lime (Pellusterts) and Calcaric Brown Lowland soils (Ustropepts) from calcareous alluvial deposit on recent plains.

5. Monoliths of main soils in the Philippines were also prepared.

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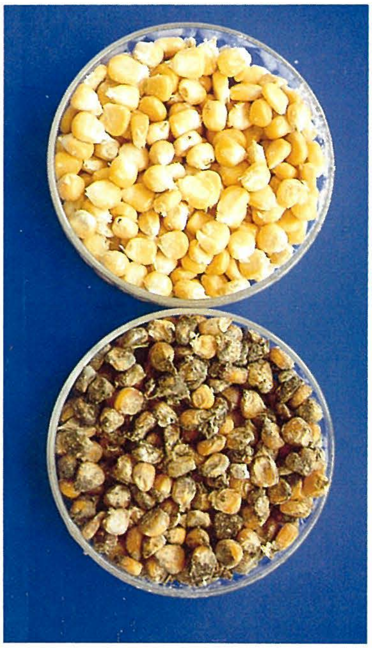
Table 1. Relationships among parent material, land form, type of climate and soil group of Red-Yellow and related soils in the Philippines.

Parent material	Land form	Soil group	Variation of soils with type of climate*			Soil name of surveyed sites
			<2 dry months	2-4 dry months	>4 dry months	Soil taxonomy
Andesite and basalt residual, old alluvial, volcanic mud flow	Hills, upper and middle terraces	Red soils	Strongly acid low activity	Strongly to moderately acid, low activity	Moderately acid	Kandihumults Kandiudults Kanhapludults Haplohumults
		Yellow soils	Strongly acid, low activity	(Strongly to moderately acid)		
	Lower terraces	Yellow soils	Moderately acid, low activity	(Moderately acid)	(Moderately acid)	
	Piedmont of volcano, terraces	Dark brown soils	Moderately acid	Moderately to slightly acid	(Slightly acid)	Paleudults Paleudalfs
Tuffaceous rocks [residual]	Hills	Red soils	Strongly acid	Strongly to moderately acid	(Moderately acid)	Haplohumults
		Brown upland soils	(Moderately acid)	Moderately acid	(Slightly acid)	Dystropepts
Metamorphic rocks residual, old alluvial	Hills terraces	Red soils	(Strongly acid)	(Strongly to moderately acid)	(Moderately acid)	
	Hills	Brown upland soils	(Moderately acid)	(Moderately acid)	(Slightly acid)	
Limestone residual, aquatic and/or eolian deposit on limestone	Limestone hills and terraces	Red soils	(Strongly acid)	Strongly to moderately acid	(Moderately acid)	Haplohumults
		Terra Rossa-like soils	Slightly acid			Paleudalfs Hapludalfs
		Terra Fusca-like soils	Slightly acid to neutral			Paleustalfs Haplustalfs
		Rendzina-like soils	Neutral to slightly alkaline			Rendolls Haplustolls
		Lithosols	Neutral			Tropothents
Calcareous sand-stone and shale residual, old alluvial, ancient dune	Terraces, ancient dune	Terra Rossa-like soils	Slightly acid			Paleudalfs Haplustalfs
	Hills	Brown upland soils	(Slightly acid)	Slightly acid	Neutral	Butropepts Ustropepts
Non-calcareous sandstone and shale residual, old alluvial	Terraces	Red soils	(Strongly acid)	Strongly to moderately acid	(Moderately acid)	Paleudults
		Pseudogley-like soils	Moderately acid	Moderately to slightly acid	(Slightly acid)	Ochraquults Albaqualfs
	Hills	Brown Upland soils	(Moderately acid)	(Moderately acid)	(Slightly acid)	
Calcareous alluvial deposit [recent alluvial]	Alluvial lowland	Brown lowland soils	Neutral	Neutral to slightly alkaline	Slightly to moderately alkaline	Ustropepts Eutropepts
		Vertisols		Neutral to slightly alkaline	Slightly to moderately alkaline	Pellusterts
Non-calcareous alluvial deposit [recent alluvial]	Alluvial lowland	Andosols	Moderately acid	Moderately to slightly acid	(Slightly acid)	Dystrandepts Eutrandepts
		Brown lowland soils	Moderately acid	Moderately to slightly acid	(Slightly acid)	Dystrandepts Eutrandepts
		Gray lowland soils	Slightly acid	Slightly acid	(Slightly acid)	Tropaquepts
		Vertisols		(Slightly acid)	(Slightly acid)	

( ) : Assumpsion *low activity : CEC of <16 me/100 g clay. strongly acid : <pH 5.0, moderately acid : pH 5.0-6.0, slightly acid : pH 6.0-6.6, neutral : pH 6.6-7.4, slightly alkaline : pH 7.4-8.0, moderately alkaline : pH 8.0-9.0

Research Period : 1987-90
Research Site : Department of Agriculture, Ministry of Agriculture and Cooperative, Thailand



Above : stored in high density polyethylene bags for 1.5 months.
Below : stored in jute bag for same period

Prevention of Aflatoxin Contamination in Maize Grains with High Moisture Content by Using Plastic Bags

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In Thailand 4 to 5 million tons of maize are produced annually and about half of the product is characterized by a high moisture content (mc 20%-30%), especially during the period from August to November which corresponds to the rainy season when the availability of sunshine for sun-drying is limited. A study was conducted to prevent *A. flavus* contamination in maize with high moisture content for a period of 10-14 days after shelling until maize is artificially dried in facilities of feed mills-exporting companies.
Key words : maize with high moisture content, aflatoxin, *Aspergillus flavus*, plastic bag

Materials and Methods

Maize with a high moisture content (Suwan-Ⅰ, mc 36.9%, 90 days after planting) was harvested and immediately shelled and packed (35kg) in jute bags. High density polyethylene bags 45 μm in thickness were used as single or double lining inside jute bags. Bagged grains were then stored for 60 days at ambient temperature and humidity (25-30℃, 75-95% RH). There were five treatments as follows ; *C*: Jute bag as control, *SR* : Jute bag with single plastic bag inside, *SM* : Jute bag with single plastic bag inside plus 2% (v/w) methanol (99.5% purity). Methanol was simply poured on to the maize in the bag, *DR* : Jute bag with two plastic bags inside, *DF* : Jute bag with two plastic bags of which the top end was folded in. Every plastic bag was tightly closed with a string except for *DF* to expel the air in the bag as much as possible. The outer jute bag was finally sealed by knitting with a string. *A. flavus* was not inoculated. During storage for two months about 2kg of maize was sampled several times and was used for further studies.

Results and discussion

In the control 3 days after shelling, 68% of the kernels were already infected. But thereafter the infection rate decreased and after 10 days there was little infection with *A. flavus* although every kernel was infected by some kinds of fungi. After 2 weeks the infection rate again increased until the end of the experimental period, presumably due to the competition among various kinds of microorganisms. However, the number of *A. flavus* fungi isolated from the maize kernel surface continuously increased during that period (Table 1). During storage the microflora in cereals changes with the environmental conditions, mainly the air humidity. Kernels in the control were heavily contaminated with various kinds of mold and lost their commercial value after 3-5 days. No yeast was detected at all after 10 days.

	0 day	3	5	7	10	14	21	30	42	60
Cont										
DRBC	0	9×10^3	6×10^4	9×10^5	1.9×10^6	1.3×10^6	1.6×10^6	1.2×10^6	7.5×10^5	9.0×10^6
YE	7.5×10^6	1×10^8	4×10^7	2.1×10^3	0(low)	0	0	0	0	0
DR										
DRBC	0	0	0	0	0	0	0	0	0	0
YE	7.5×10^6	3.3×10^6	1.9×10^7	1×10^7	1×10^7	1.3×10^5	1.3×10^5	6.8×10^5	1.9×10^6	1.4×10^4

Table 1.
Number of *A. flavus* fungi/g maize (20g/100ml dist. water, mc : 36.9%)
DRBC : *A. flavus*, YE : yeast
＊ DR was selected as it was deemed to be representative

There was little infection with *A. flavus* in the maize kernels in any of the plastic bags throughout the experimental period. Aflatoxin content in maize was analyzed after drying (mc 12%) and the results are shown in Table 2. In control, aflatoxin was not detected until 10 days but thereafter the content increased rapidly from 217 ppb (14 days) to 1395 ppb (30 days) and 977 ppb (60 days) (Fig 1). The toxin appeared rather late, presumable due to the extra-high initial moisture content of maize (36.9%).

On the other hand, aflatoxin was not detected even after 60 days in maize in plastic bags. *A. flavus, Fusarium, Penicillium, Aspergillus, Rhizopus, Botryodiplodia,* and other fungi were isolated from maize immediately after harvest. After 3 or 5 days a small number of *Fusarium* and *Penicillium* fungi were still detected. However, after 10 days no fungi were detected in all the maize in plastic bags. Oxygen consumption and CO_2 production by maize with a high moisture content may have inhibited the growth of these fungi. Fungi are known to be sensitive to oxygen concentration. Although the hermeticity of high density polyethylene film is not very high, it was noteworthy that even in such a plastic bag molds failed to grow after a short period of storage and that no *A. flavus* organism at all were detected in the *DF* bag which was not tightly closed but only folded in (Table 2).

A weak sour smell was detected in maize in plastic bags after three days and the smell became stronger during

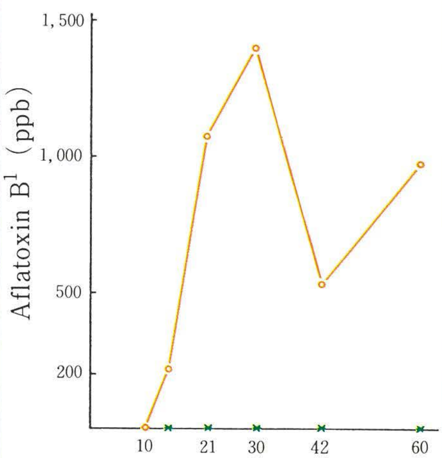

Fig 1.
Aflatoxin contamination of wet maize (mc 36.9%) after shelling

o — o in jute bag x — x in plastic bag

the storage. However, after maize was dried at 80℃ (moisture content less than 15%) the smell became less pronounced. In the *SM* bag the smell became attenuated. The pH value of stored maize was found to decrease by 0.90 in the first five days (4.90 to 4.0). The decrease of the pH value suggests the production of acid by microorganisms in maize. The changes in the microflora should be further analyzed to determine especially those of lactic acid bacteria, yeast and *Bacillus* in relation to their inhibitory activities on fungi.

Maize with a high moisture content (mc 36.9%) was successfully stored for more than 2 months without deterioration or decay. Practical advantages offered by the described procedure for the developing situations can be itemized as follows : 1) effectiveness in controlling aflatoxin production, 2) the safety and simplicity in application, 3) no requirements for special skills or equipment, 4) reusable bags costing little to small farmers, while applicable to large scale operations as well.

The disadvantages are as follows : Iron hook to pick up the jute bags can not be used. Sampling kernels from every jute bag to check the quality is difficult. Sour smell occurs if maize is stored for a long time.

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Table 2.
Infection of high moisture maize with *A. flavus* and Aflatoxin contamination during storage in HD polyethylene bag (initial maize mc : 36.9%).

		0 day	3	5	7	10	14	21	30	42	60
Cont	a	2/83	136/200	46/185	11/200	5/200	130/200	60/200	153/200	188/200	141/200
	b	ND	ND	ND	ND	ND	217	1067	1395	528	977
	c	36.9	33.6	32.1	24.1	21.5	21.0	16.6	18.7	18.1	16.2
SR	a	2/83	0/11	0/12	0/1	0/0	0/0	0/0	0/0	0/0	0/0
	b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	c	36.9	38.1	38.2	39.0	38.5	40.2	38.9	38.8	39.1	39.0
SM	a	2/83	0/4	0/14	1/5	0/0	0/0	0/0	0/0	0/0	0/0
	b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	c	36.9	34.1	39.7	39.1	40.2	40.1	36.9	30.0	39.5	39.8
DR	a	2/83	0/31	0/4	4/5	0/0	0/0	0/0	0/0	0/0	0/0
	b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	c	36.9	37.2F	38.4	38.5	38.4	39.3	35.8	39.4	38.9	38.9
DF	a	2/83	0/8	0/8	1/1	0/0	0/0	0/0	0/0	0/0	0/0
	b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	c	36.9	39.3	38.5	38.9	38.8	37.6	35.3	38.9	38.8	39.7

a : infection (kernel with *A. flavus*/kernel with other fungi) per 200 kernels,
b : aflatoxin B_1 (ppb) c : moisture content (%) SR, SM, DR, DF : see text

Utilization of Oil Palm Trees as Ruminant Feed

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Research Period : 1987-90
Research Site : MARDI (Malaysian Agricultural Research and Development Institute), Malaysia

In Malaysia, oil palm, with a cultivated area of 1.8 million hactares, is one of the main commodites of the country along with rubber.

The oil palm plantations are regenerated every 25 years and scrapped wood from the field is totally discarded. It is anticipated that the amount of residual dry matter from the trunks and fronds of old trees may reach annual values of 7 and 1.4 million tons in the latter half of the 1990s. The quality of oil palm trunk is not suitable for construction material or pulp due to the high moisture and low cellulose content, and softness. Furthermore, fronds pruned for harvesting fruit bunches are also discarded in the field without any use.

It is important, therefore, to develop a method for the effective utilization of these materials for useful purposes. Against this background, the possibility of utilizing oil palm trees as ruminant feed was considered.

Key words : oil palm, trunk, ruminant, feed, nutritive value

Old oil palm trees

Chipped oil palm trunk

Vascular bundles parenchyma

Materials and Methods

In the first part of this study, the chemical composition and in vitro digestibility of oil palm trunks and fronds (leaflets and petioles) were analyzed by the enzymatic method devised by Abe et al.

Subsequently, the effects of steaming and alkali treatment on the in vitro digestibility of the trunks and fronds were examined.

In vivo tests were, then, carried out to determine which treatment would be most effective with improving the rate of digestibility of the trunk constituents for Kedah-Kelantan bulls. In addition, comparative studies were carried out on the digestibility rate of the vascular bundles and parenchyma tissues with sheep.

Series of experiments were conducted to analyze the effect of long-term use of oil palm trunk (OPT) constituents as ruminant feed. Comparative studies were carried out to determine the body weight gain, voluntary intake and meat quantity and quality in Australian Commercial Crosses (ACC) steers which were fed ad libitum on 30% (dry matter basis) of ensiled OPT, NaOH-treated OPT and rice straw for a period of 8 months.

Results

The lignin content of the trunk wood was found to be low while the content of soluble sugars was high (Table 1), which indicated the high potential for improvement of the digestibility by chemical or physical treatments.

Steaming and NaOH treatment showed a high rate of in vitro digestibility of the trunks in comparison with leaflets and petioles.

The highest rate of in vivo digestibility was achieved by steam treatment followed by alkali treatment, ensilage and absence of treatment (Table 2).

Optimum conditions for the steam treatment were 30% moisture content, pressure of 12.5 kg/cm^2 for a period of 7 minutes and for the alkali treatment 7% of NaOH content (dry matter basis).

Even when the parenchymal tissues were not subjected to any treatment, they could be utilized as an adequate source of feed and energy.

There were no significant differences among the treatments in the average daily body weight gain which was approximately 0.7 kg (Fig. 1). Ensiled OPT group showed the lowest voluntary intake, which reflected the

Table 1.
Chemical composition of oil palm trunks and fronds

	Crude protein	Crude fat	Soluble sugars	Cellulose	Hemi-cellulose	Lignin
	% on dry matter basis					
Trunks	3.2	0.6	11.6	34.0	35.8	12.6
Fronds						
leaflets	14.5	3.2	6.5	16.6	27.6	27.6
petioles	1.9	0.5	12.6	31.7	33.9	17.4

Table 2.
Apparent digestibility of oil palm trunks subjected to various treatments and rice straw.

NDF : Neutral detergent fiber,
ADF : Acid detergent fiber

	Dry matter %	Organic matter %	NDF %	ADF %	Digestible energy Kcal/g
Oil palm trunks					
Untreated dried	42.7	38.1	25.9	49.1	1741
Steamed	54.6	50.0	32.0	70.1	2576
NaOH-treated	54.2	47.1	38.0	70.2	2320
Ensiled	50.5	48.2	60.3	96.9	2104
Rice straw	35.1	28.2	36.7	12.2	1728

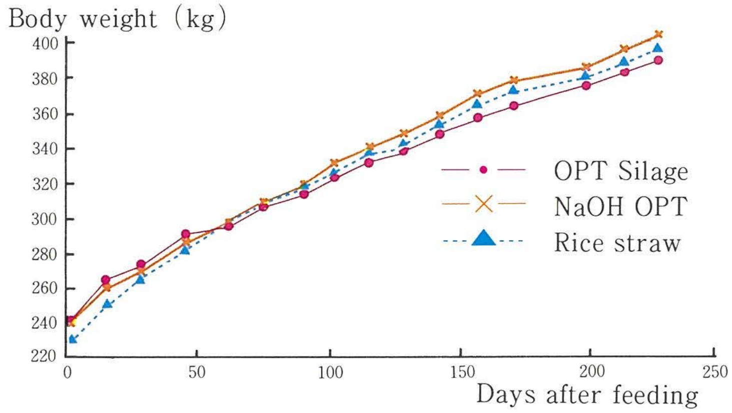

Fig 1.
Average growth rate of the steers fed with OPT or rice straw

highest feed efficiency among the treatments. Analysis of viscera and carcass composition in slaughtered animals confirmed that the use of these materials did not exert any adverse effect on the organs of the animals. Moreover, no significant differences in meat quality and quantity among the treatments were detected.

Discussion

It was confirmed in this study that OPT could become one of the promising roughage sources in Malaysia. However, the constraints on the use of OPT as economical ruminant feed include the cost of processing such as felling, chipping, treatment, transportation, and storage. The cost of processing OPT depends on many factors such as the location where OPTs are harvested, processed and fed to animals, the facilities and machines used, or the processing methods. Further investigations on economical methods of processing of OPT are required. Moreover, since OPT per se is deficient in crude protein, minerals, or vitamins, suitable combination with other economical feeds should be promoted.

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Research Period : 1988-90
Research Site : College of Forestry, University of the Philippines, Philippines

Estimation of Drought Tolerance Relating to Survival of Tropical Tree Species

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The ecophysiological characteristics of component tree species which are essential for the design of rational reforestation and agroforestry systems in the tropics have not been well documented. Among the environmental factors, water is the most important as it affects the establishment and growth of tree species. Especially drought in the dry season is the main factor preventing the establishment of seedlings that have been planted or have germinated in areas with a tropical monsoon climate. Unless the seedlings survive in the dry season, reforestation and agroforestry will not succeed. In this study we examined the drought resistance of leaves and seedlings of 15 tropical trees and discussed the relationships among the drought resistance, leaf morphology and ecological characteristics of the tree species.

Key words : drought resistance, survival, tropical tree species, dry season, leaf phenology.

Table 1.
Specific survival time of leaves cut from various tropical tree species. Vapor pressure gradient is 10 mm Hg.

Species	Specific survival time (Hrs.)	Water consumption after stomatal closure (cuticular transpiration) ($10^{-4} \cdot mg \cdot cm^{-2} \cdot s^{-1}$)	Water availability from stomatal closure to appearance of drought damage ($mg \cdot cm^{-2}$)
Shorea almon	0.43	3.06	0.468
Gliricidia sepium	0.88	4.52	1.436
Shorea polysperma	1.25	1.09	0.490
Anisoptera thurifera	2.15	1.15	0.889
Shorea contorta	2.27	3.73	3.047
Gmelina arborea	2.54	3.14	2.875
Tectona grandis	2.76	4.96	4.925
Eucalyptus deglupta	3.34	2.01	2.415
Dipterocarpus gracilis	3.37	0.99	1.201
Hopea foxworthyi	5.18	1.07	1.994
Swietenia macrophylla	5.20	2.06	3.858
Parashorea malaanonan	5.55	1.74	3.477
Acacia mangium	6.82	2.23	5.476
Acacia auriculiformis	7.57	1.79	4.880
Vatica mangachapoi	11.06	0.80	3.185

Material	Specific survival time (Hrs.)	Cuticular transpiration (10^{-4} · mg · cm^{-2} · s^{-1})	Available water (mg · cm^{-2})
Leaf	7.57	1.79	4.880
Seedling	19.15	2.65	18.273

Table 2.
Comparison of specific survival time between a leaf and a whole seedling of *Acacia auriculiformis*.

Materials and Methods

Specific survival time or Ausdauer (Pisek and Winkler, 1953) is an index of drought resistance directly related to plant survival. Specific survival time is computed from the values of cuticular transpiration (*Ec*) and available water (*Wav*) in the plant and refers to the duration between the time of stomatal closure and the appearance of the first signs of injury due to desiccation

$$\text{Specific survival time} = Wav/EC \cdots\cdots\cdots\cdots (1)$$

Survival time is measured in hours and indicates how long after stomatal closure the leaves of a plant species can remain intact without supply of water, for a given evaporative capacity of the air. We determined the critical relative water content of leaves to estimate the content of available water, using leaves dried at different rates. we determined the transpiration rate and water deficit at stomatal closure by a gravimetric method. We conducted similar experiments for whole seedlings of *Acacia auriculiformis* to estimate the degree of overall drought resistance. In addition, we observed the morphology of leaf sections, using a microscope and micrometer.

Results and Discussion

Specific survival time of leaves calculated by Eq. (1) is shown in Table 1. The survival time was shorter than 3 hours in *Shorea almon, Gliricidia sepium, S. polysperma, Anisoptera thurifera, Shorea contorta, Gmelina arborea* and *Tectona grandis*. It was longer than 6 hours in *Acacia mangium*, *A. auriculiformis* and *Vatica mangachapoi*.

The group with a short survial time included deciduous species for reforestation (*G. sepium, G. arborea* and *T. grandis*) and evergreen dipterocarp species. These deciduous species shed leaves partially or completely during the dry season (Fig. 1.) The abscission of the leaves is an effective mechanism for reducing the transpiring surface to avoid drought stress. It appeared that the deciduous species for reforestation can survive in the dry season due to

Fig 1.
Flowering *Gliricidia sepium* trees which lose leaves in the dry season.

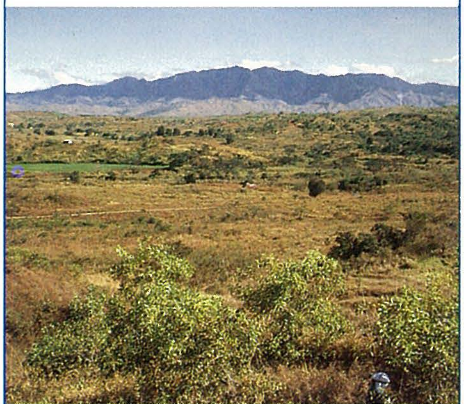

Fig 2.
Degraded forest land in the dry season in which evergreen *Acacia auriculiformis* trees were planted (front).

the abscission of leaves. On the other hand, the evergreen dipterocarp species cannot be used as reforestation species for bare land due partly to the low drought resistance of the leaves. These species planted in bare land may be damaged or killed by drought during the dry season.

The species with a long survival time were evergreen tree species. Since the leaves display a high drought resistance, these species can retain most of their leaves even in the dry season (Fig. 2).

Since only the shoot was used, this method does not measure the overall drought resistance of whole plants (Levitt, 1972). The drought resistance of whole plants was affected by the water reserves in plant and drought tolerance of non-assimilatory organs as well as drought resistance of leaves. The survival time calculated for whole seedlings was 2.5 times longer than that of leaves (Table 2). The longer survival time of whole seedlings was attributed to the larger water reserves of the seedlings.

Regarding the morphology of the leaf section, there was a close relation between the leaf thickness and critical relative water content. Namely, thicker leaves tended to be more tolerant to water deficit.

To evaluate the survival ability of tree species under dry conditions, information on drought resistance of leaves as well as on leaf phenology, drought tolerance of non-assimilating organs, water reserves and water-absorbing ability from soil as well as drought resistance of leaves is essential.

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