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Method for the Evaluation of Drought Tolerance in Cowpea

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Research Period : 1990-91
Research Site : International Institute of Tropical Agriculture (IITA), Nigeria

Since cowpea is one of the most drought-tolerant crops, it is widely intercropped with millet or sorghum in the Sudan savanna of Nigeria. However, due to the erratic rainfall in the area, especially at the beginning and the end of the rainy season, a higher tolerance to drought is needed to secure stable yield and increase the area cultivated with the crop. The development of a simple and efficient method for the evaluation of drought tolerance is a prerequisite for the breeding of drought-tolerant varieties of cowpea. Two methods were tested at the IITA Kano-Substation which is located in the Sudan savanna in Nigeria in cooperation with the IITA scientists.
Key words: cowpea, drought tolerance, soil moisture

Materials and Methods

Evaluation in the field

About 900 cowpea accessions kindly supplied by the Genetic Resources Unit of IITA were planted in the field in the dry season. To secure germination and early growth, the field was watered every morning as evenly as possible for about 2 weeks. Thereafter, the seedlings were left unwatered for about 3 months before evaluation. No precipitation was recorded during the experiment.

Evaluation using potted plants

Based on a preliminary evaluation in the field, 5 groups of cowpea accessions, ranging from highly tolerant to highly susceptible ones, were selected for a pot experiment. Each group consisted of 5 accessions, hence 25 accessions were selected in all. Small pots about 10cm in diameter with 600g of dry soill were prepared. Each accession was sown in 9 pots and the seedlings were grown with enough water for about 2 weeks in a glass house. Then they were subjected to water-stress for about 2 weeks under 3 levels of soil moisture, i.e. 5, 3 and 2 % (on a fresh weight basis). Every morning the pots were weighed using an electric balance and watered to meet the levels of soil moisture mentioned above. After the treatment, all the pots were supplied with enough water for 3 days to enable the plants to recover from drought. Then each plant was scored by observation on the basis of the following criteria. Highly tolerant(5): not affected at all, tolerant(4):

Fig.1.
Evaluation standard for drought tolerance of potted cowpeas
5: highly tolerant, 1: highly susceptible

primary leaf(pl) slightly yellowish, first trifoliate leaf(ftl) not affected, medium(3): pl. dead, ftl. actively recovering, susceptible(2):pl.dead, ftl. weakly recovering, highly susceptible(1): dead (Fig.1). Scores were averaged among 3 pots of the same treatment.

Results and Discussion

1. In the field evaluation, a wide range of drought tolerance was observed, which suggested that it may be possible to breed highly tolerant cultivars. Some accessions died soon after the termination of daily watering, while some were able to grow and develop pods (Fig.2). Thus, a large number of accessions could be evaluated in three months in the field in the dry season . However, this method is associated with some shortcomings. Evaluation was affected by the competition for soil moisture, that is, by the comparative tolerance of adjacent lines. It was also affected by the amount of residual moisture which differed from location to location. Field evaluation in the dry season, therefore, may be recommended for rough screening of a large number of materials.

Fig.2.
Field evaluation of drought tolerance in the dry season

Fig.3.
Pot evaluation of drought tolerance at three levels of soil moisture

2. Twenty five accessions were evaluated using potted plants kept under controlled soil moisture. The stress resulting from the exposure to a moisture level of 5 % was not severe enough to enable to discriminate the tolerance among the accessions (Fig.3). The stress resulting from the exposure to a moisture level of 2%, on the other hand, was too severe to discriminate the tolerance, because almost all the accessions died. Thus the exposure to a moisture level of 3 % was considered to be the optimum stress required for the discrimination. The score for the tolerance to the exposure to 3% ranged from 1.0 to 4.7 with the largest variance for the score among the accessions (Table 1). The correlation coeficient between the

evaluation in the field and that using potted plants at a soil moisture level of 3% was highly significant (r=0.663**).

Table 1.
Evaluation of drought tolerance in potted plants at three levels of soil moisture

Accession (TVu No)	Soil moisture			Remarks
	5%	3%	2%	
11982	5.0	4.7	1.0	4
14914	4.7	4.7	1.0	5
11979	5.0	4.0	1.7	5
9167	5.0	4.0	2.0	3
6914	4.3	3.7	1.0	5
7841	4.5	3.0	1.0	5
59	5.0	2.7	1.0	2
7381	3.7	2.7	1.0	3
8715	3.0	2.7	1.0	3
8713	5.0	2.3	1.0	5
433	5.0	2.3	1.0	4
928	5.0	2.0	1.0	4
127	5.0	2.0	1.0	1
85	5.0	2.0	1.0	4
7878	3.0	2.0	1.0	2
760	3.0	2.0	1.0	2
8885	4.3	1.7	1.0	4
7426	3.0	1.7	1.0	3
8365	4.7	1.3	1.0	2
7778	3.7	1.3	1.0	1
9357	3.0	1.3	1.0	2
12355	4.7	1.0	1.0	1
7758	3.7	1.0	1.0	3
8401	3.0	1.0	1.0	1
8048	3.0	1.0	1.0	1
Variance	0.70	1.25	0.05	

Remarks: Evaluation during field trial

3. The significant correlation observed between the evaluation in the field (unlimited rhizosphere) and that in pot experiments (limited rhizosphere) suggested that organs other than roots played an important role in the mechanism of drought tolerance.

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Decomposition Characteristics of Tropical Peats

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There are more than twenty million hectares of lowlands covered with peat soil in tropical Southeastern Asia, of which about 2.4 million hectares are located in Malaysia. In spite of considerable constraints on crop growth, there has been an increasing demand to develop peat soil swamps for agriculture, because peat soils occur fairly extensively near the coastal lowlands which are also the most developed and densely populated areas in Malaysia. Agricultural land use on peat soil in Peninsular Malaysia increased from 179,000 ha in 1966 to 314,000 ha in 1984.

As the soil originates from partially decomposed plant biomass it inherits the intrinsic nature of biomass, i.e., the decomposition by microorganisms. Since more than 98 percent of the original peat soil consists of organic matter, the decomposition enhances surface subsidence which is irreversible and is the most difficult constraint to overcome in peatland agriculture. The determination of the surface subsidence of Malaysian arable peatland after the initial stage of extensive subsidence ranges from 2 to 4 cm per year. In addition, the decomposition is associated with the emission of carbonaceous gases such as carbon dioxide, which is an important factor contributing to the global greenhouse effect.

Studies on the microbial decomposition of tropical peat soils have been carried out at MARDI Jalan Kebun Station since 1990, under a collaborative project between MARDI and TARC.

Key words: peat soil, microbial decomposition, decomposition kinetics, surface subsidence, greenhouse effect gas, carbon dioxide

Materials and Methods

The rate of the decomposition of peat soil organic matter was determined by aerobic incubation at a constant temperature of 35℃, and by fitting the data into computer simulation of a two-compartment exponential decay model which consisted of two fractions, i.e. easily-decomposable labile fraction (C_1) with a rate constant (k_1) ($time^{-1}$) and non-easily decomposable fraction (C_2) with a rate constant (k_2) (Murayama et al., 1990);

Research Period : 1988-91
Research Site : Malaysian Agricultural Research and Development Institute (MARDI), Malaysia

Photo 1.
Measurement of CO_2 flux from peat soil surface

Photo 2.
Annual surface subsidence of 3cm has been observed since 1958

$Y_t = C_1 \exp(-k_1 t) + C_2 \exp(-k_2 t) \cdots\cdots (1)$

where Y_t is the residual amount at time t.

The effects of the addition of $CuSO_4$ and $ZnSO_4$ and hydroxy-aluminium chloride solution (Hydroral C50B, Taki Kagaku Co. Ltd) on peat decomposition were examined by incubation and kinetics analysis.

The decomposition of peat materials *in situ* was determined by the measurement of the emission of carbon dioxide (CO_2) from the soil surface in a selected field using a gas collection chamber and gas chromatograph equipped with a thermal conductivity detector (TCD).

The collection of peat soil samples and the measurement of the CO_2 flux were carried out for various land use types at MARDI Jalan Kebun, Tanjong Karang (Northwest Selangor Forest Reserve), MARDI Pontian and Pontian Kecil (Western Johore Comprehensive Agricultural Development Scheme Area).

Results and Discussion

Malaysian tropical peats were found to decompose at a slower rate than Japanese temperate peats, presumably due to the larger C/N ratio and lower content of carbohydrates in the woody peats of Malaysia compared with the grassy peats of Japan.

The easily-decomposable fraction (C_1 of eq. (1)) of the tropical peats was very small, accounting for less than 1 percent, and equation (1) was substituted for the following single compartment exponential decay model.

$Y_t = Y_0 \exp(-kt) = 1 \exp(-kt) \cdots\cdots (2)$

Rate constants (k) obtained by the model for the tropical peats ranged from 0.24 to 3.88 ($\times 10^{-4} \cdot day^{-1}$), which are equivalent to an annual decomposition rate of 0.88-13.2 percent and to a half-life of 4.9-78.6 years. The decomposition rate was well correlated with the soil acidity (pH); the higher the pH, the higher the decomposition rate (Fig. 1). Peat soil with a lower C/N ratio, and/or higher ash content tended to decompose at a higher rate.

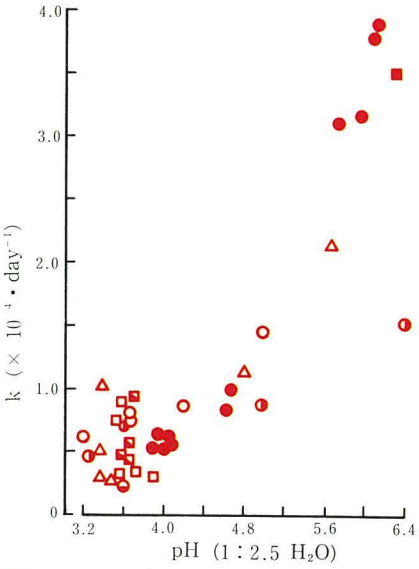

Fig. 1.
Relationship between soil acidity (pH) and rate constant (k) of the decomposition of peat under aerobic conditions at 35℃
○ ◑ ● ● : Jalan Kebun peats
△ : Tanjong Kerang peats
□ ◪ ■ : Pontian peats

The decomposition rate increased with the amendment of the soil acidity of peat by the addition of calcium carbonate and/or ground magnesium limestone which are currently used for the improvement of the soil acidity in peat soil agriculture. On the other hand, effects of standard amounts of NPK fertilizers on the decomposition rate were not observed by the incubation of the peat soils sampled from the experimental field of maize (*masmadu*) where urea was used for N source, triplesuperphosphate for P source and muriate of potash for K source.

Although the addition of heavy metals such as Cu, Zn

and Al compounds retarded the decomposition of peat organic matter by 4-10 percent, the effect was not always dependent on the amount of materials added. The most obvious and consistent effects were observed when hydroxy-aluminium was added. As a result, the soil pH decreased and peat organic matter was transformed into chelating complexes leading to the stabilization of organic matter. Aluminium polymer appears to be a suitable additive for inhibiting the decomposition of peat materials.

Carbon dioxide flux from the soil surface is another index to indicate the rate of decomposition of peat soil organic matter. The CO_2 flux ranged from 5.8 to 30.3 mmole$\cdot m^{-2}\cdot h^{-1}$, equivalent to 0.7-3.6 kg Carbon ($ha^{-2}\cdot h^{-1}$). The CO_2 flux tended to increase with the soil pH (Fig.2) and/or with the increase of the ash content and was not correlated with the soil temperature measured in the range of 24.5 to 33.5℃.

Annual surface subsidence (h cm) of arable peatland caused by the decomposition of peat soil organic matter was calculated based on a new mathematical model (Murayama et al., 1992);

$$h = 105.2 \cdot \alpha \cdot d^{-1} \cdot \theta^{-1} \cdots\cdots (3)$$

where α is the CO_2 flux (mmole$\cdot cm^{-2}\cdot h^{-1}$), d is the bulk density ($g\cdot cm^{-3}$), θ is the carbon content of peat soil. The relationships between α, θ and d are illustrated in Fig. 3. For Malaysian peat, α ranged from 0.0006 to 0.0030, θ from 0.45 to 0.60 and d from 0.080 to 0.35. More than half of the surface subsidence of arable peatland is due to the microbial decomposition of peat organic matter. For example, the annual surface subsidence (h) by the microbial decomposition in a MARDI maize field amounted to 1.55 cm when α: 0.00203, θ : 0.551 and d: 0.250, which contributed to 52 % of the whole annual subsidence of 3 cm at this site. The corresponding values for a vegetable field at MARDI Pontian Station were found to be 1.64 cm (h) and 66 %.

By postulating that the average CO_2 flux from peat decomposition associated with oil palm cultivation is 0.0010(mmole$\cdot cm^{-2}\cdot h^{-1}$) for an acreage of 150,000ha, 0.0015 for the cultivation of other dryland crops (24,000 ha), and nil for padi, rubber, coconut which grow under submerged or very wet conditions, the total annual amount of CO_2 emitted from the peatlands of Malaysia is 1.96×10^6 tons carbon. This amount is equivalent to 0.0039 % of the annual global soil organic matter decomposition, based on the reports of Sjors (1980) and Andriesse (1988) who stated that tropical peat decomposition

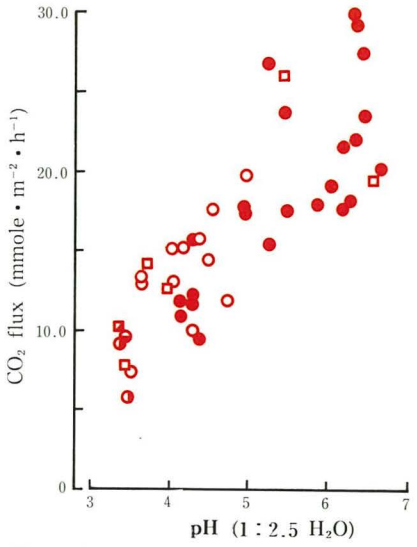

Fig. 2.
Relationship between soil acidity (pH) and CO_2 flux from the soil surface of peatland
●●●● : Jalan Kebun, Selangor
■■ : Pontian, Western Johore

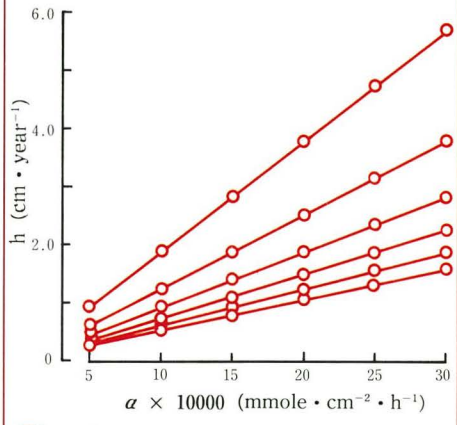

Fig. 3.
Annual surface subsidence (h) of arable peatland in relation to CO_2 emission from the soil surface (α) and bulk density of peat (d)
d; 1: 0.100, 2: 0.150, 3: 0.200, 4: 0.250, 5: 0.300, 6: 0.350
Carbon content of peat (θ); 0.550

associated with agricultural activities contributes to the increase of CO_2 in the atmosphere.

The amendment of the strong soil acidity of peat soil is a prerequisite for agriculture in peatland. However, in the two experiments, (a) the aerobic incubation-kinetics analysis, and (b) measurement of CO_2 flux from the soil surface of a maize (*masmadu*) field using different levels of liming, the use of ground magnesium limestone was found to stimulate the decompositon of peat organic matter.

Even though it is difficult to completely suppress the microbial decomposition of peat soil materials *in situ*, near sustainable agriculture could be possible if the decomposition rate were to be reduced. Agricultural utilization of peatland inevitably accelerates the decomposition and subsequent loss of peat soil materials. Peatland agriculture is particularly meaningful in the concept of "LISA" (Low Input Sustainable Agriculture) for the preservatrion of the peat soil resources for future generations.

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Nitrogen Transformation in Upland Soils without Tillage in Brazil

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Research Period : 1991
Research Site : The Botucatu College of Agronomy, Brazil

Since no-tillage cropping system has been regularly introduced to Brazil since 1982, it has prevailed in the southeastern districts of Brazil, mainly in the State of Parana. The prevalence of the no-tillage cropping system is attributed to the fact that soil erosion can be significantly prevented by this practice. In addition, no-tillage cropping is effective for stabilizing the production of upland crops and saving the cost of fertilizer and fuel, etc. However, it has been pointed out that excessive accumulation of nutrients and organic matter in the surface layers may occur in the soils not subjected to tillage. Although the growth and yield of the upland crops are considered to be affected by the changes in the vertical distribution of nutrients and organic matter, the influence has not been fully elucidated. Among the processes whereby the nutrients in soil are supplied to crops, nitrogen transformation is of primary importance. Thus, we determined the N transformation rates in the soils not subjected to tillage by using the ^{15}N-ammonium isotope dilution technique, and compared the results obtained in the soils which had been subjected to tillage by conventional methods. We attempted to determine the spatial and temporal changes in the N transformations in soil associated with the introduction of the no-tillage cropping.

Key words: no-tillage cropping, nitrogen transformation, ^{15}N-ammonium isotope dilution method, Brazil

Materials and Methods

This study was conducted as a joint project between the State University UNESP (Universidade Estadual Paulista) and TARC.

The soils used in the experiments were collected from the experimental fields of the UNESP which were located at Botucatu (abbreviated as BT) and São Manuel (SM) in the State of São Paulo. The other soils were collected from São Gotardo (SG) in the State of Minas Gerais. The soils are classified as terra rossa (BT), sandy latosol (SM), and yellow latosol (SG), respectively, and their chemical properties are listed in Table 1. In each field, wheat and soybean were grown in the plot without tillage and the

Table 1.
Chemical properties of the soils*

Soil	pH ($CaCl_2$)	Organic matter (%)	CEC (meq/100cm³)	Maximum water holding capacity
BT	5.7	3.4	12.3	55.1
SM	5.5	1.8	4.8	33.5
SG	6.2	4.9	9.8	53.5

* These values were obtained from the soils at a depth beween 5-25 cm in the conventional plot.

adjacent plot with conventional tillage every year. Non-tillage cropping was continued for 6 years in the BT and SM plots, and for 4 or 10 years in the SG plot. The amount of fertilizer applied and cultivation method except for the tillage method before sowing were the same in the plot without tillage and the conventional plot. Samples of the BT and SM soils were taken before wheat was harvested (from 6 September through 10 September), and those of SG after the crops were harvested (on 25 September). The soil samples were sieved (<2mm), and used without drying.

The rates of N transformation were determined by using the ^{15}N-ammonium isotope dilution technique (Kirkham & Bartholomew, 1954; Nishio et al.,1985). To each soil, 0.02 mgN g^{-1} of ^{15}N-ammonium sulfate (21.1 atom % ^{15}N) was added. The moisture content of the soils was adjusted to 50% (BT,SM) and 60% (SG) of the maximum water-holding capacity of the soils, respectively. The soils were incubated at 25℃ (BT,SM) or at 22℃ (SG), and the temporal changes in the contents of ammonium, ^{15}N-ammonium, and nitrate were determined.

Results and Discussion

Temporal changes in the ammonium and ^{15}N contents in the BT samples are shown in Fig. 1. The content of ammonium and ^{15}N-ammonium decreased with time in all the samples. The rate of decrease in the surface soil (0-5 cm) of the plot without tillage was much more rapid than that in the other soils. Although the temporal changes in ammonium and ^{15}N content were almost identical in the 0-5 cm and 5-20 cm layers of the conventional plot, the changes in the subsurface soils (5-20cm) of the pot without tillage were slower than those of the other soils. The content of nitrate in all the soils increased, and the rates of nitrate production in the soils were the same as the decreasing rates of ammonium (data were omitted).

The rates of N transformation determined by the ^{15}N-ammonium isotope dilution method (i.e. rates of mineral-

ization, immobilization, and nitrification) in the BT and SM soils are shown in Table 2. Among the values obtained, the rates of BT-N-1 and SM-N-1 were remarkably high. The mineralization rate of BT-N-1 and SM-N-1 was about 3 times higher, respectively, than that of the same layer in the control plot (BT-C-1 and SM-C-1). The rates of immobilization and nitrification were simultaneously high in BT-N-1 and SM-N-1. Although the rates of BT-C-2 (or SM-C-2) were similar to those of BT-C-1 (SM-C-1), the rates of BT-N-2 (SM-N-2) were much lower than those of BT-N-1 (SM-N-1). All the samples taken at a depth below 25 cm showed low activities for N transformation. The same trend as that of the N transformation was observed for the distribution of organic N. The amount of organic N in the surface soils of the plot without tillage was larger compared with that of the other soils. However, the rate of N transformation in the surface soils in the plot without tillage was more enhanced than expected by the increace in the amount of organic matter.

The rate of N transformation in the surface layer of the plot without tillage increased with the duration of no-tillage cropping (only the values of the mineralization rates are shown in Fig.2). The content of organic matter increased with the duration of no-tillage cropping, too. However, in the subsurface layer (5-25cm), the N mineralization rate decreased rapidly, while the organic N content did not decrease significantly.

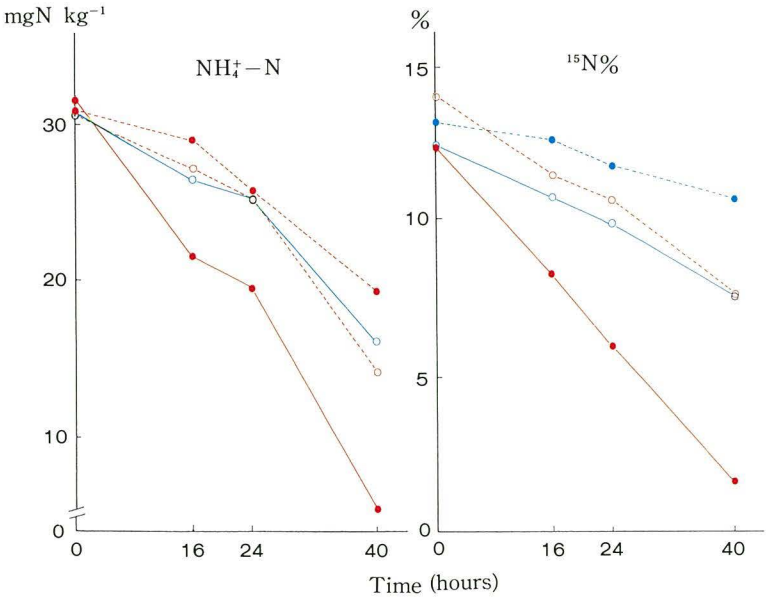

Fig. 1.
Temporal changes in ammonium and ^{15}N content in the BT soils
Symbols: O—O ; 0-5 cm, conventional plot, O····O ; 5-20 cm, conventional plot, ●—● ; 0-5 cm, plot without tillage, ●····● ; 5-20 cm, plot without tillage

Table 2. Nitrogen transformation rates in soils*

Location	Plot	Depth	N transformation rates			Organic N	Abbreviation
			minerali.	immobili.	nitrifi.		
		(cm)	(mg N kg^{-1} d^{-1})			(g N kg^{-1})	
Botucatu	conventional	0- 5	6.5	4.5	7.9	1.91	BT-C-1
		5-20	8.3	5.9	7.7	1.89	BT-C-2
		25-40	1.6	1.2	1.4	1.05	BT-C-3
	no-tillage	0- 5	21.1	19.2	17.2	2.17	BT-D-1
		5-20	3.0	3.5	4.5	1.74	BT-D-2
		25-40	1.0	0.6	1.2	0.92	BT-D-3
São Manuel	conventional	0- 5	3.5	4.0	2.2	0.51	SM-C-1
		5-25	6.2	5.9	2.0	0.52	SM-C-2
		25-40	0.7	1.1	1.0	0.51	SM-C-3
	no-tillage	0- 5	11.9	11.8	7.8	0.76	SM-D-1
		5-25	0.9	1.7	2.2	0.54	SM-D-2
		25-40	1.7	1.9	1.5	0.53	SM-D-3

* The amount of $(^{15}NH_4)_2SO_4$ (21.1 atom% ^{15}N) added to soils was 20 mgN kg^{-1}.
The soils were incubated at 25℃.

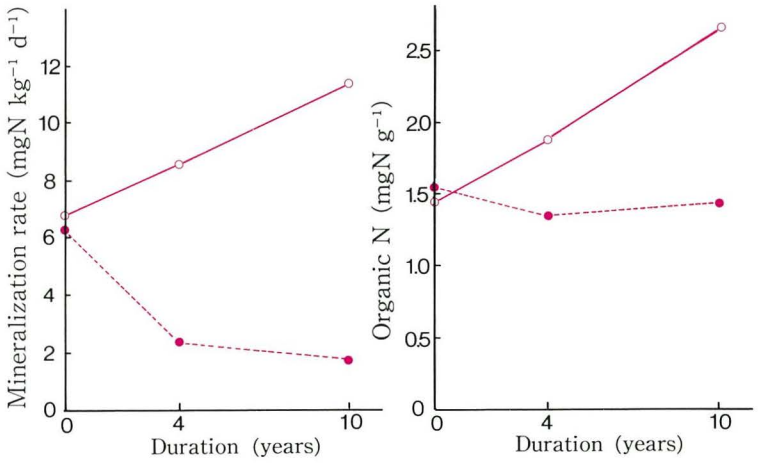

Fig. 2.
Changes in N mineralization rate and organic N content in the SG soils with the duration of no-tillage cropping
Symbols: O—O ; 0-5 cm (surface layer), ●·····● ; 5-25 cm (subsurface layer)

In the soils without tillage, the depth at which N transformation was enhanced was less than 5 cm at most. Under this layer, the rate of N transformation gradually decreased year by year. It is assumed that the major portion of N absorbed by crops in the fields with continuous absence of tillage was supplied by the surface layer shallower than 5 cm. Although the yield of crops usually decreased in the initial years after the onset of no-tillage cropping, the yield recovered and became stabilized after that period. From the viewpoint of N supply to crops over a long period of time, there appear to be no significant problems regardless of whether N-cycling in the soil-plant

system takes place within the surface layer of the soil.

The increase in the rate of N transformation in the surface layer of the soils not subjected to tillage exceeded that of the soil organic matter. In contrast, the N transformation rate declined in the subsurface layer, though the amount of soil organic matter did not decrease appreciably. These facts suggest that the ratio of easily decomposable organic N to the total amount of N increased in the surface layer and decreased in the subsurface layer. Since the decomposition of easily decomposable organic matter in the surface layer proceeds rapidly, steady application of organic matter to the soil without tillage may be necessary to maintain the soil fertility.

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Biotype Composition of the Brown Planthopper Populations in and around the Muda Area, West Malaysia

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Research Period : 1988-90
Research Site : Malaysian Agricultural Research and Development Institute (MARDI) Alor Setar Station, Malaysia

The development of host plant resistance is one of the most efficient measures to alleviate insect pest problems in the integrated pest management strategy. The use of insect-resistant varieties is an attractive method for farmers to control the brown planthopper (abbr. BPH), *Nilaparvata lugens*, one of the major insect pests of rice, because it is simple and inexpensive in comparison with other methods of control. A large number of BPH-resistant varieties have been bred and distributed in South and Southeast Asia mainly by IRRI. However, some of these varieties lost their resistance within a few years after the introduction due to the appearance of virulent BPH biotypes, or never developed field resistance from the beginning of introduction. This is a serious problem for the use or distribution of resistant varieties, because it takes a long time (several years or more) to breed a new resistant variety. It is, therefore, important to determine what kind of biotypes occur in the area before resistant varieties are introduced or what kind of biotypes are likely to appear after the introduction of the resistant varieties. Such studies should contribute to the enhancement of the

Table 1.
Date of collection and tested generation of BPH populations

Collection site	Population	Date of collection	Tested generation (collected gen. = 0)
Muda area	Bukit Besar	Jul. 12, 1989	1
	Sungai Padang	Jul. 12, 1989	1
	Telok Chengai	Jul. 26, 1989	1
	Pendang 1	Jul. 31, 1989	1
	Pendang 2	Jul. 31, 1989	1
	Telok Chengai	Jan. 10, 1990	4
	Kampong Kandai	Feb. 12, 1990	2
	Bukit Besar	Jun. 26, 1990	0
	Telok Chengai	Jul. 26, 1990	3
Outside Muda	Kuala Muda	Aug. 2, 1989	1
	Seberang Perak	Dec. 8, 1989	1
	Seberang Perak	Jul. 5, 1990	4

effectiveness of resistant varieties.

This study aims to identify the biotypes of BPH collected in and around the Muda irrigation scheme, the largest rice production area in Malaysia, by comparing the feeding activity of the adult female on several standard resistant rice varieties.

Key words: *Nilaparvata lugens*, biotype, resistant variety, Malaysia

Materials and Methods

A total of 12 BPH populations was collected from various rice fields from July 1989 through July 1990. Each population was kept in a rearing cage with rice seedlings (variety 'TN1'). Female adults in the offspring generation were used for the test as shown in Table 1.

We detected the biotype composition of BPH populations by comparing the amount of honeydew excreted by the female adults on 5 standard resistant varieties; 'Mudgo' (harboring the resistance gene *Bph 1*), 'ASD7' (*bph 2*), 'Rathu Heenati' (*Bph 3*), 'Babawee' (*bph 4*) and 'TN1' (lacking resistance genes). We adopted the ninhydrin treatment method or the bromocresol green treatment method (Fig. 1) to detect the amount of honeydew. It was assumed that the more BPH sucks the sap from the rice plant, the more it excretes honeydew. Thus, the quantity of honeydew excreted by an insect is considered to be an index of feeding activity on each standard variety. We measured the area of the honeydew spots on treated filter paper (Fig. 2). The area was considered to reflect the feeding activity of the female on each variety.

Results and Discussion

The amount of honeydew excreted by the Telok Chengai population collected in July 1989 (abbr. T. Chengai 8907) on 5 varieties is shown in Fig. 3 as an example. Some females on ASD7 and Mudgo excreted as much honeydew as those on TN1, unlike on Rathu Heenati and Babawee. These insects are considered to be biotypes capable of attacking ASD7 or Mudgo. Considerable individual variations in the amount of excretion were observed even on the susceptible variety TN1 as well as on the resistant varieties.

To compare the excretion among the populations, we standardized data; namely, 1) half replicates with a larger amount of excretion were selected from each variety in each population, 2) the average excretion on TN1 in each population was rated as 100 and the relative excretion of each insect was calculated. This index was used for the comparison of the feeding activity on each variety among

Fig. 1.
Bromocresol green method to detect BHP honeydew
A feeding chamber with a filter paper treated with bromocresol green was placed at the base of the plant. One adult female was caged in this chamber for 2 days. The honeydew excreted by the female was collected on the filter paper

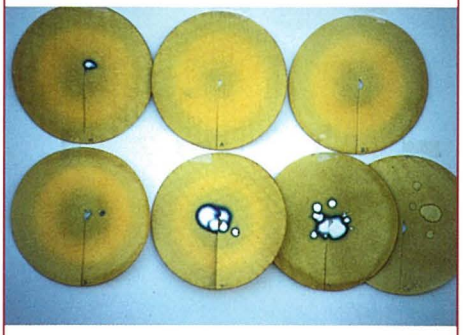

Fig. 2.
Honeydew spots on the filter paper treated with bromocresol green
When the honeydew drops on the filter paper, blue spots appear due to the change of pH that can be distinguished from the plant sap (bottom, extreme right)

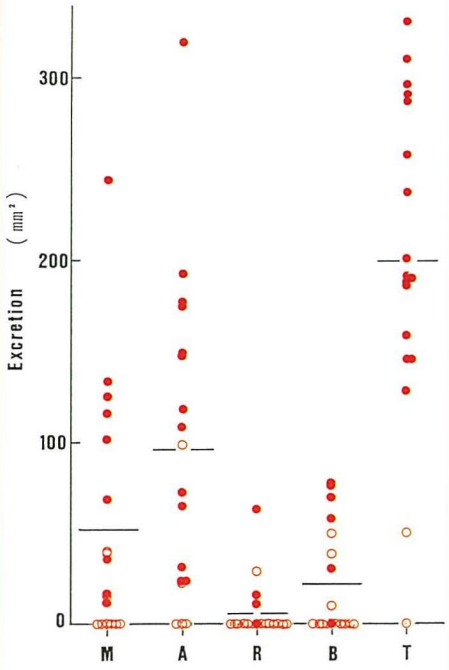

Fig. 3.
Amount of honeydew excreted by females of 'T. Chengai 8907' population
Each circle represents the excretion by one insect. Black circles indicate the insects which survived during the test and white circles the insects which died during the test
M: Mudgo, A:ASD7, R: Rathu Heenati, B: Babawee, T:TN1, — : average.

the populations.

The relative excretion of 12 populations on 5 varieties is presented in Fig. 4. The females in the K. Kandai 9002 population on Mudgo, and the females in the B. Besar 8907, T. Chengai 9001 and K. Kandai 9002 populations on ASD7 excreted a comparable amount of honeydew to that on TN1 (not significantly different by U-test). Other populations excreted a significantly lower amount of honeydew on resistant varieties than that on TN1 ($P<0.01$ by U-test). Among the 4 resistant varieties, insects generally showed a relatively higher feeding activity on ASD7 followed by Mudgo, while the feeding activity on Rathu Heenati and Babawee was low.

These results indicate that the BPH populations in and around the Muda area consist of a mixture of biotypes: some insects are able to attack the varieties harboring *Bph 1* and/or *bph 2* genes, though the extent of virulence is limited.

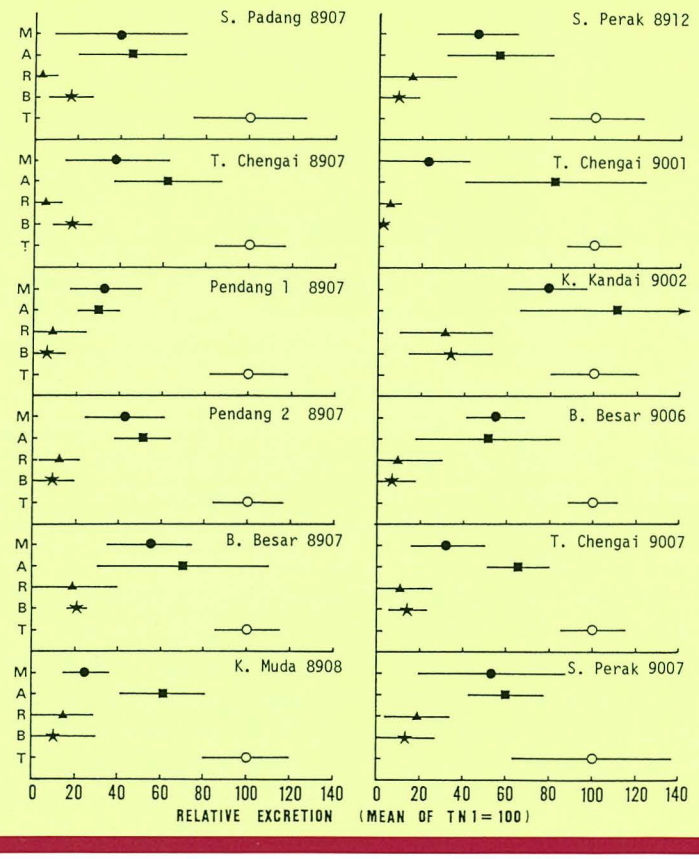

Fig. 4.
Relative excretion of 12 BPH populations collected in and around the Muda area on 5 varieties
Each symbol indicates the mean and the range of standard deviation
M: Mudgo, A:ASD7, R: Rathu Heenati, B: Babawee.

Cultural Control of the Root-Knot Nematode, *Meloidogyne javanica*, Attacking Mungbean in Thailand.

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Research Period : 1986-92
Research Site : Department of Agriculture, Ministry of Agriculture and Cooperative, Thailand

Mungbean is an important pulse crop not only for local consumption but also for exportation in Thailand as well as other Asian countries. In the past 10 years, the yield of mungbean (about 333,000 tons per year) has remained stationary although efforts to increase the yield were made in the field of breeding, methods of cultivation, plant protection, etc. in Thailand. Recently mungbean damage caused by Java root-knot nematode, *Meloidogyne javanica* (Fig.1,2), has often been detected in crop cultivation areas as one of causes of yield reduction in mungbean. Methods of control of the nematode should be developed not only by using nematicides which are associated with the contamination or destruction of the environment and ecosystems. Therefore, the effect of cultivation of antagonistic or non-host plants and organic material application to soil on the population density of the root-knot nematode was examined as a part of the development of cultural control.

Key words: mungbean, *Meloidogyne javanica*, antagonistic plants, organic materials, Thailand

Fig.1.
Females of *Meloidogyne javanica* invading roots of mungbean

Fig.2.
Mungbean roots attacked by *Meloidogyne javanica*

Materials and Methods

Cultivation of antagonistic or non-host plants to nematodes

Plants or crops antagonistic to the root-knot nematodes, which included African marigold (*Tagetes erecta*), upland rice, guinea grass and maize were planted in Wagner pots (1/2000a) with soil containing a high density of *Meloidogyne javanica* after mungbean cropping. After two months, the nematode density of the soil in which the test plants were cultivated was estimated in comparison with that in the soil under continuous cropping of mungbean. Yield of mungbean plants after cultivation of different kinds of crops was also determined to analyse the effect of various methods of crop cultivation on the growth of mungbean.

Application of organic materials

One kilogram each of compost (urban waste compost), dry cow dung or dry silkworm feces (1500 kg per 10 a in conversion) was mixed with soil heavily contaminated with *M. javanica* in large square concrete pots (80cm

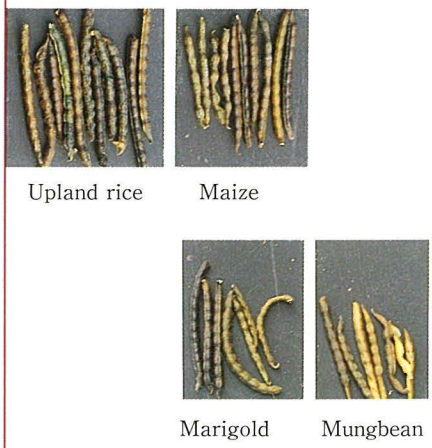

Fig.3.
Comparison of pods of mungbean cultivated after planting of antagonistic or non-host crops

length of each side and 50cm depth), and a plot treated with 10 g chemical (Furadan) and a non-treated plot were prepared as control. The mungbean seeds were sown 2 weeks after the mixture of organic materials and 10 plants each were cultivated for the experiment. The nematode density before and 3 months after the application of organic materials was compared to determine the effect of the respective materials on the nematode density. Yield of mungbean was also compared between the material application and the nematicide application or the absence of treatment.

Results and Discussion

Cultivation of antagonistic or non-host plants to nematodes

Population density of *Meloidogyne javanica* after cultivation of such non-host plants as guinea grass, upland rice and maize decreased remarkably and fairly after cultivation of an antagonistic plant, African marigold, whereas the nematode population increased in the case of continuous cropping of mungbean. Yield of mungbean during the cultivation of non-host plants was higher than that under mungbean continuous cropping but the mungbean yield under African marigold cultivation was not significantly different (Table 1, Fig. 3). Reduction of the population of *M. javanica* after cultivation of antagonistic or non-host plants was considered to be caused by the death and/or lack of development of the nematodes invading the roots of these plants and also by the absence of host.

Table 1. Effect of cultivation of antagonistic or non-host crops on population density of *Meloidogyne javanica* infesting mungbean and yield of the crop

Crops cultivated	No. of nematodes per 100 g soil		Rate of increase (%)	Weight of mungbean seeds (g)
	Before cultivation	After cultivation		
Guinea grass	290	7	2.4	104 a
Upland rice	218	9	4.1	96 ab
Maize	195	13	6.7	82 b
African marigold	157	43	28.1	45 c
Mungbean	247	340	137.7	31 c

Each value is a mean of 5 replications.

Table 2. Effect of application of organic materials on population density of *Meloidogyne javanica* infesting mungbean and yield of the crop

Organic materials and chemical	No. of 2nd stage larvae of *M. javanica* per 100g soil		Rate of increase (%)	Weight of seeds (%)
	Before application	After application		
Compost	343 (48)	144 (324)	42	80 a
Silkworm feces	270 (110)	165 (596)	61	72 a
Cow dung	249 (37)	205 (167)	82	46 b
Nematicide (Furadan)	121 (133)	736 (42)	608	28 b
No treatment	317 (78)	1272 (94)	401	24 b

Each value is a mean of 5 replications.
Figures in parentheses indicate the number of free-living nematodes.

Application of organic materials

The population density of *M. javanica* after application of organic materials significantly decreased in comparison with the nematode population before application and it increased in the case of chemical application or absence of treatment. Population density of free-living nematodes on the contrary, increased by the application of organic materials and decreased by chemical application or the absence of treatment (Table 2).

The results of this experiment indicated that the application of organic materials, especially compost or silkworm feces increased the density of free-living nematodes including some kinds of nematodes preying upon pest nematodes and probably altered the physical and chemical properties of soil resulting in an environment unsuitable for the nematodes. Organic materials presumably produce some substances that kill the pest nematodes in soil in the process of their decomposition.

It is concluded that studies on the introduction of antagonistic or non-host plants into the cultivation system and practical use of organic materials in fields are very useful for the control of pest nematodes as well as for the maintenance of the fertility of arable soil.

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Echinochloa Weeds in Peninsular Malaysia

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In recent years, labour shortage and rising labour cost have motivated many farmers in the Muda area, Malaysia to switch from transplanting to direct seeding culture of rice. The changes were closely related to the development of new cultivars and widespread adoption of combine harvesters. The changes have resulted in dramatic shifts of weed infestation and distribution.

Key words: *Echinochloa*, weed control, wet-seeding rice culture, Barnyardgrass

Observation Sites

Main site is the Muda irrigation scheme (about 100,000 ha, double cropping, wet or dry-seeded rice) and its adjacent rainfed areas, Seberang Perai (double cropping wet-seeded or transplanted) and Kerian (deep water area, transplanted). Direct seeding culture spread rapidly in the 1980s. The climate of the area is intermediate between that of tropical rain forest and tropical savanna. Mean annual temperature is around 27℃, annual rainfall ranges from 1,500 mm to 2,500 mm.

Materials and Methods

Two thousand specimens of over one hundred weed species were studied in those areas. The 90 observation fields were located in the Muda area. The fields were observed 3-6 times / season from 1989 to 1991. Habitat conditions, time emergence and heading of each species were recorded. Plant characters, especially panicle characters and chromosome numbers of some species, were recorded.

Results and Discussion

Identification

Echinochloa crus-galli complex displays intraspecific morphological variations. Significant differences between the two types were as follows: short awn, open panicles, spikelets shiny for *E. crus-galli* var. *formosensis* and long awn (sometime awnless) with closed panicles for *E. crus-galli* var. *crus-galli* (Table 1). Value of one thousand seed weight of *E. oryzicola* was twice as large as that of *E.*

Research period : 1989-91
Research Site : Muda Agricultural Development Authority and Malaysian Agricultural Research and Development Institute, Rice Research Center, Malaysia

Fig.1.
Comparison of panicles of *Echinochloa oryzicola* (left) and *E. crus-galli* var. *formosensis* (right)

Fig.2.
Rice field heavily infested with *Echinochloa oryzicola* in Tikam Batu, Kedah, Malaysia

Table 1. *Echinochloa* species in Northern Peninsular Malaysia

Common name	Species name	Synonyms	Life cycle & Main habitat
Tainubie(J) Sambau(M) 2n=36	*E. oryzicola* Vasing.	*Panicum oryzicola* Vasing. *E. crus-galli*(L.) Beauv. var. *oryzicola* (Vasing.) Ohwi	Annual, Wet-seeded rice & transplanted rice fields
Himetainubie(J) Sambau(M) Barnyardgrass(E) 2n=54	*E. crus-galli* (L.) Beauv. var. *formosensis* Ohwi	*E. glabrescens* Munro ex Hook f. *E. crus-galli* (L.) Beauv. var. *kasaharae* Ohwi *E. micans* koss.	Annual, Wet-seeded rice, dry-seeded rice & volunteer seedling culture
Inubie(J) Sambau(M) Barnyardgrass(E) 2n=54	*E. crus-galli* (L.) Beauv. var. *crus-galli*	*Panicum crus-galli* L. *E. oryzoides* (Ard.) Fritsch *E. crus-galli* (L.) ssp. *hispidula* (Rets.) Honda	Annual, Wet-seeded rice, dry-seeded rice & volunteer seedling culture
Kohimebie(J) Padi burung(M) Jungle rice(E) 2n=54	*E. colona* (L.) Link	*E. colonum* (L.) Link *Oplismerus colonum* H. B. X. *Panicum colonum* L.	Annual, Volunteer seedling culture, upland rice (hill padi) & dry-seeded rice
Sambau merah(M) 2n=54	*E. stagnina* (Retz.) Beauv.	*Panicum stagninum* Retz.	Perennial, Wet-seeded rice canal & drainage
Sambau merah(M) 2n=126	*E. picta* Michel	*E. stagnina* (Retz.) Beauv.	Perennial, Wet-seeded rice

(J) : Japanese name, (M) : Malaysian name, (E) : English name

crus-galli (Table 2). *E. oryzicola* could also be distinguished from the Malaysian populations of *E. crus-galli* by the shape of the first empty glume (Fig.1). All the Malaysian populations showed the F - form (in which the lower lemma is flat and coarse). The chromosome number of *E. oryzicola* was 2n=36.

Distribution and habitat

E. crus-galli complex was widely distributed and caused heavy damage to rice in all the rice granary areas, especially the Muda and Barat Larut areas. *E. oryzicola* was distributed in Seberang Perai and Muda areas (Fig.2). *E. colona* was mainly distributed in upland rice and dry-seeded rice fields.

Ecological characters

The heading time of *E. oryzicla* was later, and that of *E. colona* was earlier than that of both varieties of *E. crus-galli*. Seed longevity of the *E. crus-galli* complex in Malaysia was shorter than in the temperate region. The two perennial species were moderately short day plants. Seeds of *E. picta* and *E. stagnina* were sterile, vegetative

Table 2. Seed characters of *E. oryzicola* and *E. crus-galli* in Malaysia

Species	1000 seed weight (g)	Spikelet length (mm)	Spikelet width (mm)	Collection site
E. oryzicola	4,338	3.70	1.83	Tikam Batu
E. crus-galli var. *crus-galli*	1,639	2.85	1.44	Muda area*
E. crus-galli var. *formosensis*	2,652	2.99	1.70	Muda area#

* : kg. Tandop, District IV, Alor Setar, Kedah

: kg. Limau Dalam

reproduction depended on propagation by sprouting of axillary buds from stolons and fragmented culms.

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Evaluation of Legume Species for Use as Green Manure Crops in the Sub-Tropics in Japan

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Tropical Agriculture Research Center

Research Period : 1987-91
Research Site : Okinawa branch, Tropical Agriculture Research Center, Japan

Some islands in Japan, which are called Nansei-shoto, are situated in the sub-tropics. Presently sunhemp (*Crotalaria juncea*) is widely cultivated as a green manure crop in a cropping system of sugarcane planted in summer. Rotation with green manure crops is important in cropping systems of upland crops including sugarcane and vegetables, in order to avoid the physical and chemical deterioration of the soil and protect the land against erosion by heavy rain in these islands, since soil organic materials are rapidly degraded under hot temperature conditions in the tropics and sub-tropics.

In order to identify legume species with a higher biomass potential that would be adapted to the soil and environmental conditions of the Japanese sub-tropics compared with sunhemp currently cultivated, we evaluated legume species whose germplasm was collected mainly during former TARC expeditions to tropical and sub-tropical countries.

Key words: legume species, green manure crops, Okinawa

Materials and Methods

Thirty five lines among twenty five tropical and three temperate zone leguminous species were preliminary evaluated for various aspects relating to green manure utilization in 1987 (Table 1). Five species were selected by the preliminary test and subsequent screening tests performed in 1988 and 1989. The biomass production of the selected five species was compared to that of sunhemp (a commercial variety imported from Africa) under various conditions of cultivation in 1990 and 1991. In all the field experiments, the tested plants were sown in early May in a field with acid yellow soil at TARC Okinawa Branch and they were harvested from late August to early September.

Usually fertilizers were not applied except for lime and phosphorus in the field evaluation test of 1988 and except for the investigation on the effect of the application of lime and phosphorus in acid soil in a pot experiment and a frame experiment. Heavy metal uptake was examined in plants which were grown on acid red soil in order to

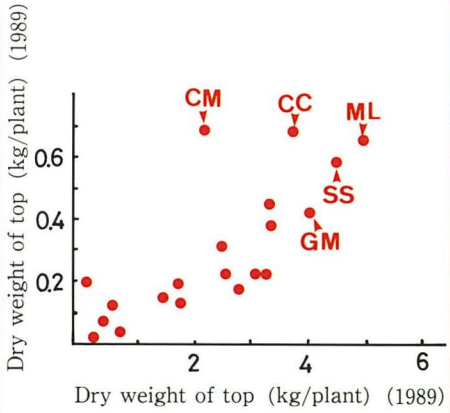

Fig.1.
Biomass production of legume species
CC: *Cajanus cajan* (Bra), CM: *Calopogonium mucunoides* (Phi), GM: *Glycine max* (Oki), ML: *Macroptilium lathyroides* (Col), SS: *Sesbania* sp. (Tai)

analyse the mechanism of adaptability of tolerant species to acid soil.

Results and Discussion

In the preliminary evaluation of 1987, the germinability of seeds and establishment of seedlings, growth under hot and dry conditions in summer, growing habit for soil covering by plant canopy, suitability for the plowing of plant bodies into the field with a tractor, possibility of propagation as aggressive weeds, tolerance to strong wind during typhoons were estimated by observation.

Moreover, through subsequent evaluations of biomass production in 1988 and 1989, five species were selected as candidates for green manure crops, as follows: one temperate zone legume, soybean (*Glycine max*, TARC Okinawa branch plant germplasm accession number OK6264, subtropical local variety collected in Miyakojima, Nansei-shoto) and four tropical legumes, i.e. pigeon pea (*Cajanus cajan*, OK6248, introduced from Brazil), phasey bean (*Macroptilium lathyroides*, OK5662 introduced from Colombia), calopo (*Calopogonium mucunoides*, OK6254 from the Philippines) and sesbania (*Sesbania rostrata*, OK5890 from the Philippines) (Fig.1). The seedlings of these species were able to survive a dry spell after germination due to their rapid early growth. Two vein type legumes, phasey bean and calopo were not severely damaged by strong wind during typhoons in summer. Leaves of pigeon pea and soybean did not become detached by the strong wind, too. Four species, except for *S. rostrata* were not seriously damaged by any insects from 1987 to 1991. Furthermore the four species except for *S. rostrata* produced a dense leaf canopy, showing that they could be used as cover crops against soil erosion by heavy rain.

They were compared to sunhemp as green manure crop in 1990 and 1991. In addition, *Crotalaria spectabilis* and velvet bean (*Mucuna pruriens*) were also evaluated as candidates for green manure crops, because the former had been used as a green manure crop in the northern Nansei islands. The latter is a green manure plant which grows well, though the leaves and veins can not be plowed into the field with a tractor until they wither after the start of winter.

In 1990, the four species selected through the previous tests were found to be superior to *C. juncea*, *C. spectabilis* in their biomass production, except for *S. rostrata*. The low biomass production of the two *Crotalaria* species was partly due to the damage by insects such as tobacco worm (*Helicoverpa* sp.) and plant bugs (*Miridae gen.* sp.) in

Fig.2.
Pigeon pea at the time of plowing into the land
Arrows show the test line (OK6248)

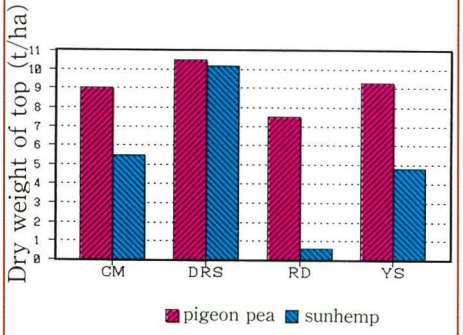

Fig.3.
Dry matter yield of pigeon pea and sunhemp in principal soil types of Okinawa
CM: soil from degraded calcareous mudstone, DRS: dark red soil originating from coral lime stone, RS: acid red soil, YS: yellow soil

Table 1. List of evaluated legume species

Species	Origin of germplasm
Cajanus cajan	Ind
C. cajan	Bra
C. cajan	Nig
Calopogonium mucunoides	Bra
C. mucunoides	Phi
Cassia sp.	Nig
Centrosema pubescens	Nig
C. sp.	Phi
Clitoria ternatea	Tan
Crotalaria zanzibarica	Unk
C. sp.	Nig
C. sp.	Tai
Desmodium intortum	Nig
Dolichos axillaris	Bra
Galactia striata	Nig
Glycine max	Oki
Glycine wightii	Eth
G. wightii	Tan
G. wightii	Tan
Lablab purpureus	Oki
Leucaena leucocephala	Oki
Macrotyloma axillare	Nig
Macroptilium atropurpureum	Tan
M. atropurpureum	Ken
M. lathyroides	Col
Medicago sativa	Jap
M. sativa	Eth
Pisum sativum	Eth
P. sp.	Eth
Sesbania rostrata	Phi
S. cannabina	Oki
S. sp.	Tai
Stylosanthes guianensis	Bra
Vigna lasiocarpa	Col
V. radiata	Phi

Bra: Brazil, Col: Colombia, Eth: Ethiopia, Ind: India, Jap: Japan, Ken: Kenya, Nig: Nigeria, Oki: Okinawa (Japan), Phi: Philippines, Tai: Taiwan, Tan: Tanzania, Unk: Unknown

1990 and 1991 and to typhoon in 1990. *S. rostrata* was also damaged by the tobacco budworm and *Eurema* sp.

In 1991, in order to compare the potential of biomass production under optimal cultural conditions for each species, two sowing dates, 18th April and 29th May were examined for all the species. Plant density which was assumed to be appropriate for each species based on the growing habit in previous field tests and plant density twice that of the former were also tested.

In 1991, biomass production of three species of pigeon pea, phasey bean and soybean was large among the five species and three control species tested. Especially pigeon pea planted at a high planting density (140,000 plants/ha) and at an early sowing date gave the highest dry matter yield of about 10.6 ton/ha (Fig.2), while the value recorded for phasey bean was 9.4 ton/ha at a high planting density (140,000 plants/ha) for either sowing date. On the other hand, although the dry matter yield of soybean was not as high as that of pigeon pea and phasey bean cultured under favorable conditions, it ranged from 5.8 ton/ha to 6.4 ton/ha regardless of the sowing date and planting density. Although velvet bean was a good cover crop whose veins and leaves grew immediately after sowing, the dry matter yield of velvet bean did not reach that of soybean like sunhemp and *C. spectabilis*.

The cultivation tests listed above were performed in fields with yellow acid soil at TARC Okinawa Branch. Adaptability of these species to different kinds of soils was examined by pot culture and frame culture. Soybean and pigeon pea grew better in acid red soil not amended with lime and not supplied with phosphorus than sunhemp and *C. spectabilis*. Furthermore, pigeon pea grew better than sunhemp in all the main soils distributed in the Nansei-shoto, which are derived from calcareous mud stone, dark red soil derived from coral lime stone, acid red soil and acid yellow soil (Fig.3). In particular, the difference in biomass production between pigeon pea and sunhemp was remarkable in acid red soil whose acidity (pH4.2) was the strongest of all the tested soils. In the tropical and subtropical acid soils, plants are deficient in phosphorus and they are exposed to toxic heavy metals released from acid soil. It was observed that sunhemp absorbed a large amount of manganese from red soil without lime application while pigeon pea as well as mungbean (*Vigna radiata*) did not absorb toxic manganese (Table 2). This ability of avoiding the uptake of manganese may account for the superiority of pigeon pea to sunhemp in acid red soil.

To identify more adaptable varieties, tests were car-

ried out with six pigeon pea varieties introduced from ICRISAT (International Crops Research Institute for the Semi-Arid Tropics) and soybean varieties introduced from Thailand and Indonesia. In the test of pigeon pea, the dry matter yield of late maturing varieties was higher than that of early maturing ones. However, we could not identify any pigeon pea varieties whose dry matter yield was higher than that of the TARC test line, OK6248 (variety introduced from Brazil). On the other hand, some improved soybean varieties used as grain legume in Thailand and Indonesia produced a larger biomass than our test line, OK6264 (Japanese sub-tropical local variety). The dry matter yield of these varieties which ranged from 7.1 to 11.9 ton/ha was comparable to that of the TARC test line of pigeon pea (Fig.4).

Thus pigeon pea and soybean show a high potential for biomass production and they display a wide adaptability to acid soil. In addition, they can be easily plowed into the field with a tractor, in particular pigeon pea which grows actively. It was assumed that both species could be substituted for presently cultivated sunhemp as new green manure crops in summer. Soybean may be a particularly suitable green manure crop because the seeds of soybean are available at a lower price than those of many legume species. The use of soybean as a green manure crop is likely to widen the range of use of this crop which is already a major food and feed crop.

References

1) Nakano, H. et al. (1990): Evaluation of tropical legume species as green manure crops. Jpn. J. Trop. Agric. 34 (Extra issue 2) ,32-33.

Table 2. Toxic heavy metal content of green manure crops

Species	Lime* application	Element (ppm)	
		Mn	Al
Pigeon pea	+	534	355
	−	107	234
Sunhemp	+	1099	140
	−	98	65
Mungbean	+	627	358
	−	98	282

*Rate of application of calcium carbonate was 4 g/4 kg red soil.

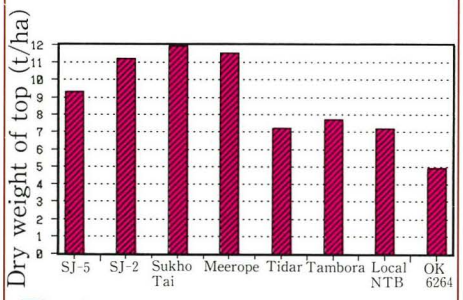

Fig.4.
Dry matter yield of soybean varieties
SJ-5, SJ-2, Sukho-Tai 1 (Thailand varieties), Meerope, Tidar, Tambora, Local NTB (Indonesian varieties), OK6264 (Local variety of Nanseishoto)

Use of Seed Pellets of Guineagrass and Tropical Legumes for Pasture Establishment in the Sub-Tropical Okinawa Islands

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1) Tropical Agriculture Research Center, 2) National Grassland Research Institute

Research Period : 1989-91
Research Site : Tropical Agriculture Research Center

Soils distributed in the sub-tropical Okinawa Islands usually designated as Maji soils are chemically and physiologically poor. These soil characteristics in addition to the climatic conditions with annual summer drought and typhoons prevent the establishment of grasslands on these islands which are mostly covered by sugarcane fields and natural grasslands with low-yielding indigenous grass and shrub species. In order to alleviate these shortcomings, it was considered that the use of seed pellets like soybean or maize seeds may enable to sow very small tropical grass and legume seeds uniformly and economically in the fields, and to apply micro-nutrients, such as Zn, Fe, and Mo which are frequently deficient in the tropical and sub-tropical soils. Therefore, we developed methods to produce seed pellets of guineagrass, which is the most promising tropical grass species in Okinawa Islands, and of 7 tropical legumes, *Macroptilium atropurpureum* cv. Siratro, *Centrosema pubescens* cv. Centro, *Stylosanthes hamata* cv. Verano, *S. scabra* cv. Seca, *Desmodium intortum* cv. Greenleaf, *D. uncinatum* cv. Silverleaf, *Neonotonia wightii* cv. Tinaroo. Also we produced seed pellets containing the micro-nutrients mentioned above and phosphorus for good seed germination. Thereafter, we developed mechanical methods for seeding these pellets for the establishment and renovation of grasslands in the tropical and sub-tropical regions.

The seed pellets mainly consisted of volcanic ash soil, which occurs over large areas in Japan. The shape was mostly spherical and size ranged from 0.8 to 1.5 cm in diameter depending on the seeds and the purposes (Fig. 1).

Key words: guineagrass, tropical legumes, seed pellet, Okinawa

Fig. 1.
Germination rate of *Stylosanthes* seed pellets put into a red Maji soil without soil covering

1. Seed pellets of guineagrass

The cultivars used for the guineagrass seed pellets were Natsukaze and Natsuyutaka which were released from Kyushu National Agricultural Experiment Station and appeared to be suitable for cultivation in the warm temperate areas and in the sub-tropical area of Japan, respectively.

The seed pellets were produced by mixing 3,000 g of volcanic ash soil passed through a 1 mm mesh net, 300 g of clay soils such as bentonite or perlite if necessary, 80 g of guineagrass seeds (germination rate; 40%), and ca. 1,000 ml of water in a mixing machine (Kneader). The mixture was put into another machine (Pelleter), to obtain tight sticks 0.5 mm in diameter. Then, the sticks were put into a "Marumerizer" (trade name) to obtain spherical pellets ca. 8 mm in diameter on a turning plate. These wet pellets were dried at 40℃ for 2 hours. Each seed pellet contained 7 to 15 seeds from which 2 or more seedlings germinated.

2. Seed pellets of tropical legumes

The same seed pellets were produced for the cv. Verano and cv. Seca of *Stylosanthes* whose commercial seeds are covered by a hard pod. The seed pellets of the other tropical legumes with various sizes, however, were crushed or cracked after drying. It was assumed that since the size of commercial seeds sold without pods may increase after the seeds were mixed with soil and water, though the pellets became smaller after drying, crushing or cracking of the seed pellets may have occurred.

We noticed that the thickness of the Greenleaf seeds, which are smallest of all, rapidly increased in water from an initial value of 0.9 mm to 1.2 mm after 3 hours (Fig. 2). The thickness of the Centro seeds, which are the largest of all, increased from 2.3 mm to 2.8 mm in 6 hours (Fig. 2). The maximal sizes in water were 1.25 and 2.95 mm, respectively.

Germination percentages of dried seeds after soaking in water from 1 to 14 hours were investigated to produce seed pellets by using soaked seeds. The germination ability of the dried seeds of the cv. Greenleaf and cv. Centro remained intact after the seeds were soaked in water even for 14 hours (Table 1).

On the basis of these results, we developed a new method to produce pellets of legume seeds. Production of the seed pellets of the two legume species without the occurrence of cracking or crushing became possible when the seeds of the cv. Greenleaf and those of the cv. Centro were allowed to soak and swell in water for 3 and 6 hours, respectively. The seed pellets of the other species could

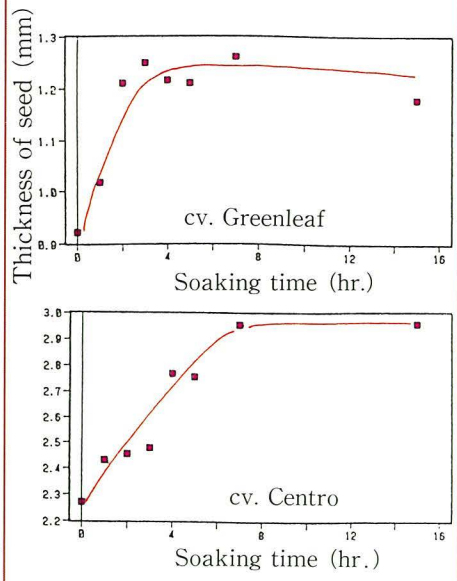

Fig. 2.
Relationship between the thickness of legume seeds and soaking time in water

Cultivar	Soaking Time (hr.)						
	1	2	3	4	5	7	14
Greenleaf	32.0	16.0	26.0	27.0	25.0	19.0	20.0
Centro	48.0	61.0	58.0	57.0	65.1	60.0	77.0

Table 1.
Germination rate (%) of the dried legume seeds after soaking in water for 1 to 14 hours

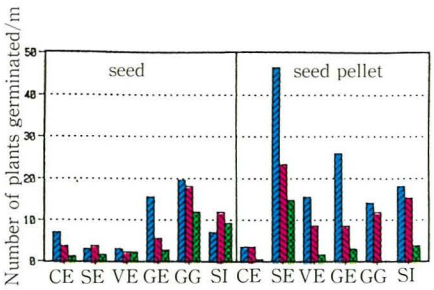

Fig. 3.
Germination rate of seeds and seed pellets sown in a red Maji soil with or without watering
CE:Centro; SE:Seca; VE:Verano; GE: Greenleaf; GG:Natsuyutaka; SI:Silverleaf
Left :Watering every 2 days
Middle:Watering once when seeded
Right :No watering

also be used after soaking the seeds in water for 3 to 6 hours. The size of the seed pellets varied depending on the seeds, that is the seed pellets of *Stylosanthes* and cv. Greenleaf were 8 mm in diameter, while those of the cv. Silverleaf and the cv. Tinaroo were 1.2 mm in diameter, and those of the cv. Centro and cv. Siratro were 1.5 mm in diameter. Each seed pellet contained 3 to 6 seeds.

3. Properties of seed pellets

The seed pellets rapidly absorbed water and their water content which depended on surrounding water conditions became maximum within 1 hour. Besides, when the seed pellets were buried in the ground, they absorbed water efficiently from the surrounding soils with a relatively low water content. The water content of the seed pellets which exceeded 20% remained at this level for 10 days when that of the surrounding soil was about 10% and the seed pellets germinated very well. The number of germinated plants from seed pellets, was larger than that from the seeds under different water conditions when a similar number of seeds with or without seed pellets was seeded on a red Maji soil distributed on Ishigaki Island (Fig. 3). This phenomenon was more obvious for the seed pellets and seeds of the cv. Seca than for the other cultivars.

4. Addition of micro-nutrients and phosphorus to the seed pellets

Addition of micro-nutrients, such as molybdenum, zinc, iron, and phosphorus to the seed pellets was possible. The addition of 5 g of $NaMoO_4$ in 3.3 kg of soil, $2H_2O$, 50 g of $Fe_2(SO_4)_3$, and 1.2 kg of phosphate to the seed pellets of the cv. Seca and cv. Tinaroo did not affect the germination rate, though the germination rate of the seed pellets of the cv. Siratro was somewhat lower than that of the control, after the addition of 100 g of $ZnSO_4$.

5. Mechanical sowing of seed pellets

Mechanical sowing of the seed pellets was carried out by using a Grain Drill Seeder at Okinawa Prefectural Livestock Experiment Station, Nakijin, Kunigami, Okinawa, and by using a linked-belt type seeder at the Okinawa Branch. As it is difficult to transport and sow the seed pellets, the use of the seeding machines listed above could be beneficial.

The seed pellets of guineagrass and tropical legumes are very effective for the establishment of grasslands on the drier, chemically and physically poor soils distributed in the tropical and sub-tropical regions.

Analysis of Rangeland Vegetation using Remote Sensing

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1) Tropical Agriculture Research Center, 2) International Center for Agricultural Research in the Dry Areas

Research Period : 1990-91
Research Site : International Center for Agriculture Research in the Dry Areas (ICARDA), Syria

Outline of the Test Site

This study was conducted in the Maragha National Range (Syria) which is located at 35°33' N, 37°41' E. (Fig. 1). Annual rainfall which is estimated to be about 200mm, is mainly distributed in winter (December-March). The growing season of vegetation occurs during the period February to April after rainfall.

A model diagram of yearly supply of food for sheep is shown in Fig. 2. The animals depend on the steppe for about 42% of the total supply. Recently, the area devoted to crop fields has expanded in the northwestern zone around the Maragha Nat. Range.

Key words: remote sensing, vegetation, rangeland, Syria

Methods

LANDSAT data

Path 173, Row 35 TM 1989/3/21, 1989/4/22, 1990/3/8, 1990/7/14, 1991/4/28

Balloon Aerial Photo System

Photographs taken from a balloon at a height of 200m (Fig. 3) were digitized by using a color image scanner, Epson GT4100.

Global Positioning System

The exact position of the area studied was determined using a Sony PYXIS G.P.S. which is a receiver of G.P.S. Satellite ultrahigh frequency signal (L band 1.57GHz). The accuracy of the measured position is about 35m on the ground.

Image Processing and GIS Software

ILWIS (Integrated Land and Watershed Management Information System), produced by ITC (The Netherlands), PC-based geographic information system with the following main functions;

Remote Sensing (Visualization, Enhancement, Classification, Geographical Correction, Image Arithmetic)

Geographic Information (Digitizing, Polygonization, Vector to Raster Conversion)

Internal Database and Interface (Table Manipulation, Database Operation)

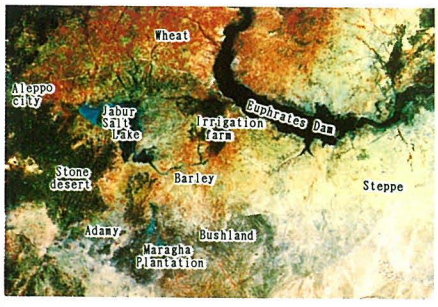


Photo 1.
LANDSAT 5 TM 1990. 3. 8.
North Syria

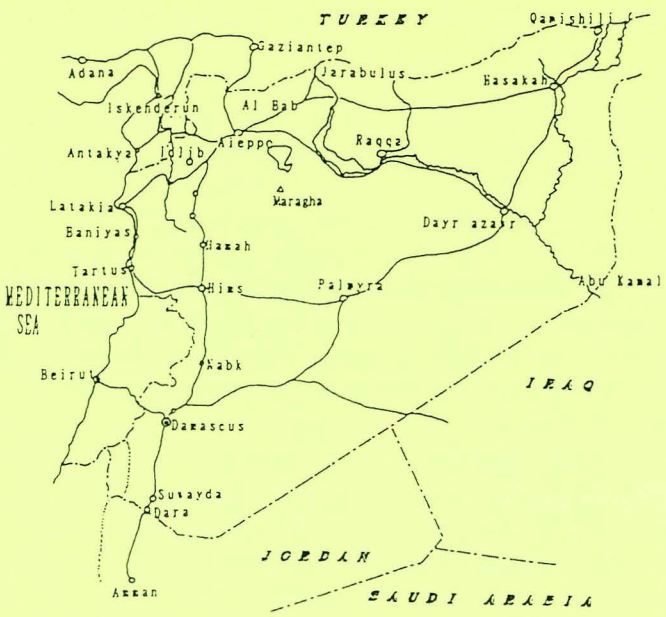


Fig. 1.
Map of Syria

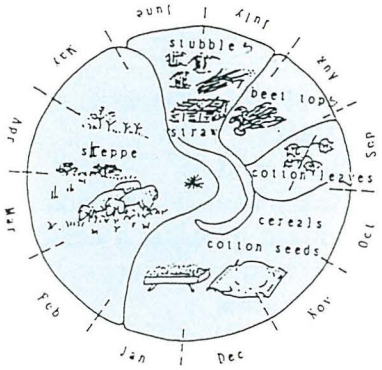


Fig. 2.
Model diagram of yearly supply of food for sheep

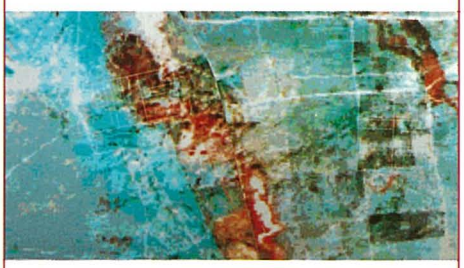


Photo 2.
Near infra-red color composite satellite imagery, Maragha, April, 1991
Plots 1-9 are Natural plots and Plots 10-18 are improved plots

Results

1. Interpretation of satellite imageries

General survey of land-use in North Syria

LANDSAT 5 TM data of 1990/3/8 were combined to infra-red false color imageries which were useful for the interpretation of the land-use and vegetational conditions. Compared with old land-use data which were interpreted by LANDSAT MSS in 1975, many crop fields were found to encroach upon grazing lands (Photo 1).

Analysis of shrub plantations

Rangelands are being improved by the cultivation of fodder shrubs, for example, *Atriplex halimus*, *A. canescens* and *Salsola vermiculata*. We can analyze the planted areas based on enlarged false color imageries in the following combinations; 4band=red, 3band=green and 2band=blue. Shrub areas give a dark color, and can be defined

clearly in the Adamy and Maragha areas (Photo 2). Plantation map, scale 1:60,000, was constructed from LANDSAT imagery.

2. Analysis of large scale aerial photographs

Species and density of planted shrubs

Photo taken from the balloon shows the difference in species. Canopy of *Atriplex halimus* is larger than that of *Salsola vermiculata*. Plant coverage percentage was estimated at 20.4 % for *A. halimus* and 4.9 % for *S. vermiculata* using aerial photos (Photo 3).

Vegetational patterns on natural grassland

Photo 4 shows the vegetational pattern on the natural plot 1. Dark color indicates the areas with dense vegetation and whitish color those with poor vegetation, due to the micro-topography, where hills appear whitish and valleys dark (Photo 4).

Line intercept observation of depressed areas

In the northern part of plot 3, there is a round depressed area, with a diameter of about 90m. The details of the vegetation were obtained from ground survey. The dominant species were *Peganum harmala*, *Hordeum bulbosum*, *Carex* sp. and *Plantago lagopus*. *Hordeum* predominated on hill sides and *Peganum*, *Carex* and *Plantago* predominated in depressed areas.

Vegetational condition of grazing trial

Balloon photographs distinctly show the vegetational condition of the grazing trials which were treated by application of superphosphate at the rates of 0, 25 and 60 kg P_2O_5/ha and stocking rates of L : low (1.2ha/sheep) and H : high (0.65ha/sheep) (Photo 5).

Discussion

Grazing intensity should be controlled according to the grazing capacity, which can be estimated based on near infra-red color imageries. Other factors involved include soil type, topography and climate. After acquisition of these data, a bio-mass map will be processed to obtain a grazing capacity map.

Acknowledgements

This study was carried out at the International Center for Agricultural Research in the Dry Areas (ICARDA, Aleppo, Syria) as a collaborative research project. The author is greatly indebted to Dr. A.E.Osman (Pasture, Forage and Livestock Program) for providing the test site for grassland trial and ground truth data. Assistance of Mr. Fahim Ghassaly (P.F.L.P.) for the operation of the balloon system and identification of plant species in the

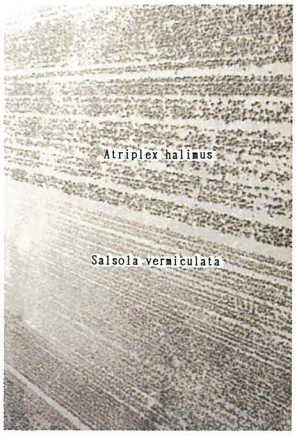


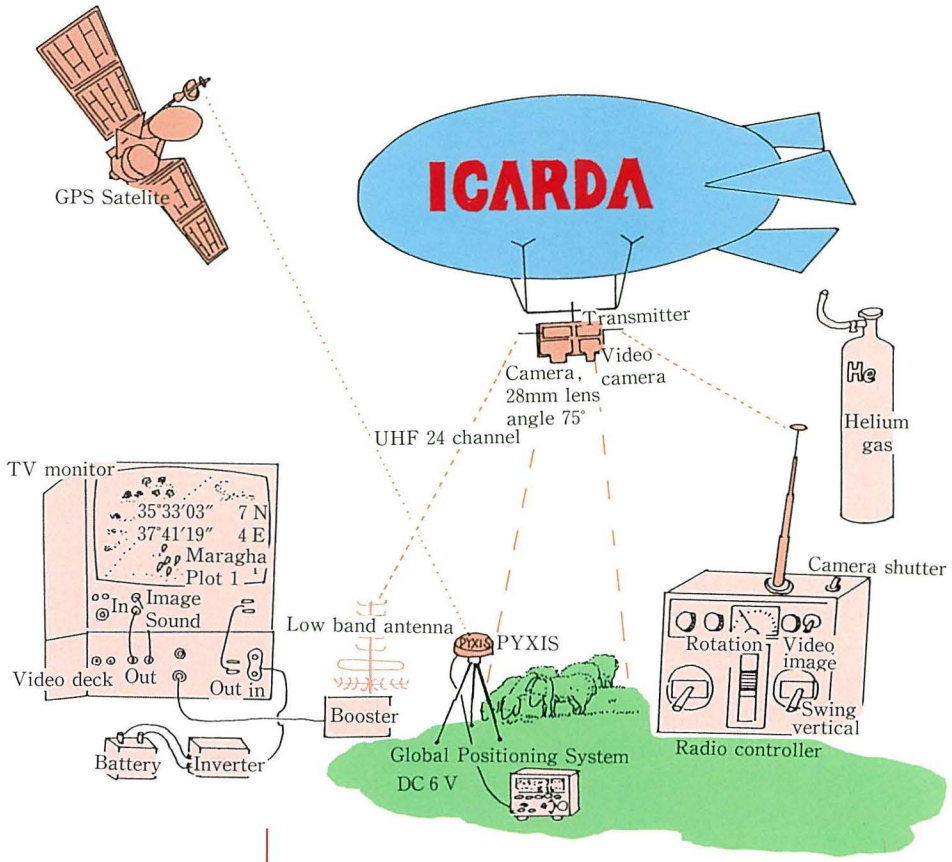
Photo 3. Improved Plot 11

Photo 4. Maragha Natural Plot 1

Photo 5.
Photograph of the Tel Hadya Grassland Trial taken from a balloon

P 0 L : Phosphate 0 kg, Low stocking rate,
P25L : 25kg, Low
P60L : 60kg, Low
P 0 H : Phosphate 0 kg, High stocking rate,
P25H : 25kg, High
P60H : 60kg, High

Fig. 3.
Balloon aerial photograph system



test field is gratefully acknowledged.

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Development of Methods for the Improvement of Seedling Establishment in Wet Seeding Rice Culture in the Muda Area in Malaysia

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1) Tropical Agriculture Research Center, 2) Tohoku National Agricultural Expriment Station, 3) MADA Alor Setar

The Muda irrigation scheme is the largest rice granary of Malaysia. In the early 1980s, as the labour shortage for transplanting became acute, some farmers in the Muda area adopted direct seeding culture to overcome this constraint. After several attempts, the direct seeded area increased exponentially, reaching 76,900 ha or 85% in the first cropping season and 56,300 ha or 58 % in the second cropping season during the three-year period from 1986 to 1988. However, direct seeding culture in the Muda area is associated with various unstable factors compared with transplanting. The objective of this study is to develop methods for the improvement of seedling establishment in wet seeding rice culture which is widely adopted both in the first and second cropping seasons.

Key words: wet seeding rice culture, tractor wheel ruts, Auger trencher, Malaysia

Research Period : 1988-90
Research Site : Muda Agricultural Development Authority (MADA), Alor Setar, Malaysia

Materials and Methods

Yield trials in wet seeding culture were carried out to identify the optimum planting density and the appropriate amount of nitrogen application from 1988 to 1990. The farm operations and seedling establishment in wet seeding culture were mainly surveyed at site A which belonged to the Muda 1 project in the second cropping season, 1988. The supplementary data for seedling establishment were collected at site B which belonged to the Muda 2 project in the same cropping season and year.

To promote seedling establishment, various methods of improvement were evaluated. 1) Drainage method through ditches formed by tractor wheel ruts ; this method was developed by Tanaka (1983) and Sawamura (1984-88). The ditches were formed by driving a tractor over the field. Interval of the wheel ruts was about 8 meter. 2) Drainage method through ditches dug with an Auger trencher (Photo 1) : a tractor-operated Auger trencher was used to dig ditches along the boundary of paddy fields under dry conditions. The depth of the ditches from the field surface ranged from 26cm to 33cm. The width was 40

Photo. 1.
Ditches dug with an Auger trencher to promote water drainage (Niplo OM-310D)

cm.

Results and Discussion

Paddy yields in the first cropping season appeared to have decreased with the spread of direct seeding culture and the shortage of irrigation water since 1984. Paddy yield in the second cropping season also seemed to have decreased or remained stationary along with the spread of wet seeding culture compared with the yields achieved in 1980 and 1981 when only transplanting was implemented. Various experiments and field surveys were carried out to identify the optimum planting density for wet seeding culture. Based on the yield decrease in the plots with less than 100 plants per m^2 and the occurrence of hopperburn caused by brown planthoppers at a high planting density of more than 200/m^2 under standard nitrogen application (80 kg/ha), the recommended planting density for stable yield ranged from 100 to 150 plants/m^2.

Furthermore, the yields in the wet-seeded plots were as high as those in the transplanted ones or even higher when the growth of the rice plants was normal (Table 1).

Table 1.
Comparison of yield between wet seeding culture and transplanting culture of rice (1989)

Treatment	Yield (kg/ha)		No. of spikelets ($\times 10^3/m^2$)	
	Cropping season		Cropping season	
	1st	2nd	1st	2nd
Wet seeding	6.7	5.8	26.4	27.3
Transplanting	5.7	5.2	23.2	23.8
Wet seeding /Transplanting (%)	118*	112^{ns}	114	115

*Significant at 5% level

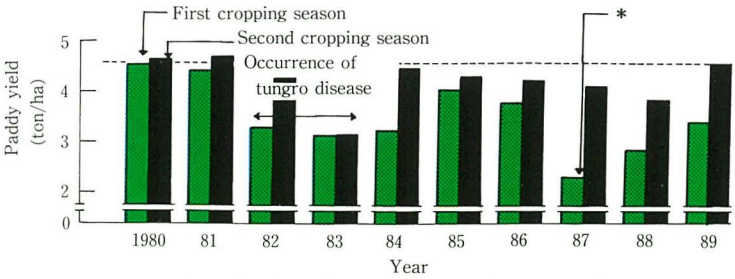

Fig. 1.
Changes in paddy yield per hectare in the first and second cropping seasons in the Muda area

*Interruption of supply of irrigation water due to water shortage in storage dams

As for seedling establishment in wet seeding rice culture in the Muda area, the total area of the fields with vacant spots when seedling establishment had failed, accounted for 6.0% or more of the entire area, amounting to about 20% of the combined area for site A, and about 35% for site B. The average number of seedlings established at site A was as high as about 160 plants per m^2 with an

average coefficient of variation of 50%. However, about 20% of the fields exhibited a low rate of seedling establishment with an average value of less than 100 plants per m^2 which resulted in a remarkable yield decrease. Low seedling establishment and vacant spots when seedling establishment had failed were observed due to poor drainage through the shallow ditches made by dragging a gunny sack filled with soil. To improve the drainage in wet-seeded fields the use of diches formed by tractor wheel ruts was tested. We confirmed that this method was very effective for the drainage of the surface water in the fields and for the promotion of seedling establishment (Fig. 2). As many farmers were concerned about the increase in unplanted areas; it was suggested that ditches dug by tractor wheel ruts be constructed at intervals of around 15 m.

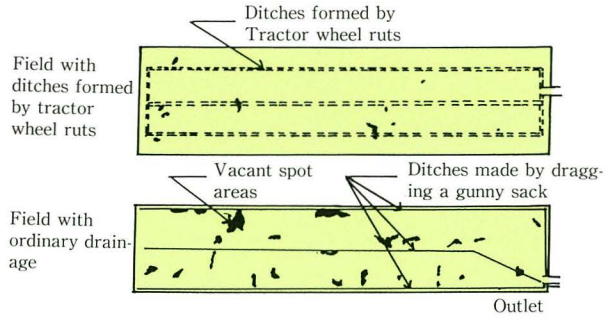

Fig. 2.
Comparison of frequency of vacant spot areas where seedling establishment had failed between field with ditches formed by tractor wheel ruts and field with ordinary drainage (2nd cropping season, 1990)

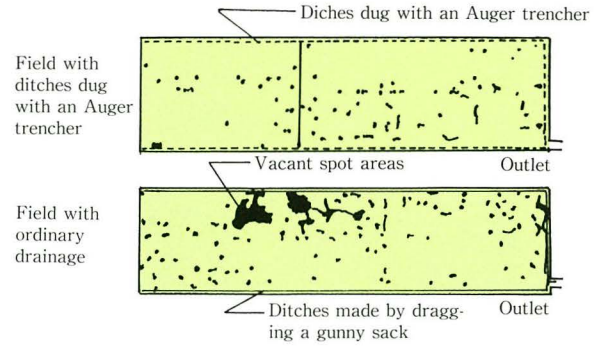

Fig. 3.
Comparison of frequency of vacant spot areas where seedling establishment had failed between field with ditches dug with an Auger trencher and field with ordinary drainage(2nd cropping season, 1990)

On the other hand, the method of drainage of surface water by using the Auger trencher was more effective and could be applied in small unplanted areas (Fig. 3). Furthermore, this method may be suitable for fields where the drainage canal and irrigation canal are separated by a long distance such as in the Muda area.

The drainage methods in wet seeding culture were highly evaluated as they enabled to achieve stable and high yields in the Muda area.

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Establishment of Tropical Agricultural Research Optical Disk Information System (TRODIS)

Daisuke SUZUKI[1] and Mitsuo SUZUKI[2]
1) Tropical Agriculture Research Center (Present address: National Agriculture Research Center), 2) Tropical Agriculture Research Center

Research Period : 1991
Research Site : Tropical Agriculture Research Center

A large amount of information on agriculture and forestry in tropical and sub-tropical countries is not formally published. However, documents such as maps, figures, leaflets, etc., have been collected by researchers. This information should be stored for further use.

A database was constructed for these documents using a text digitizing information system consisting of a laser disk with a higher filing capacity so as to retrieve, edit and print easily the information.

Key words: text digitizing information system, database, classification code, network

Classification and Categories

The type and structure of the input data were analyzed before the introduction of an optical disk system and the function was checked in the hardware for the data base construction.

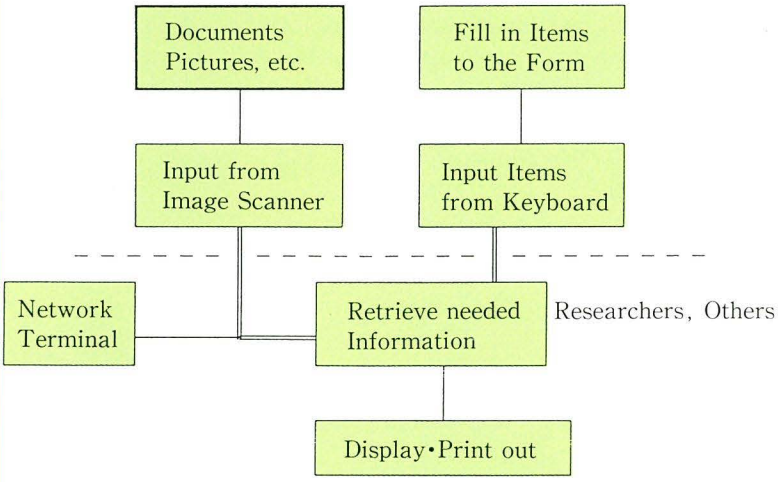

Fig. 1.
Flow chart of retrieval system

As a result, the system enabled to input ANK (Alphabetical, Numerical and Kana) characters and fulfilled a network function due to the large amount of literature in Roman characters at TARC. A local area network was thus constructed.

The classification code was determined before the

data were input. As a result, the organization classification consisted of 12 items, research fields of 173 items, crops of 752 items, countries of 198 items, document forms of 29 items, and document location codes of 11 items based on TARC original codes.

The retrieval items were also determined. As a result, 14 items were adopted including the title (within 30 characters), sub-titles(15), free key words (writhin 10 items, one item within 10 characters), and bibliography references (20), etc. based on the style of the data.

A 5.25 inch optical disk can file 17,000 sheets for A4 paper size when the digitizing accuracy is 200 dots per inch. The maximum size of input data sheet is A3 and the reading speed of the scanner is 2.5 seconds per sheet when the paper size is A4 and digitizing accuracy is 200 dots per inch.

Fig. 2.
A view of the hardware

Results and Discussion

The network system can be used with a personal computer connected to the LAN for the text digitizing information system. The user manual and the list of classification key words were developed to register a document in the system.

References

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Manual for Practical Detection of Ten Rice Viruses in Plants and Insects

Toshihiro OMURA[1], Koichi ISHIKAWA[1], Hiroyuki HIBINO[1], Tsuneo TSUCHIZAKI[2], Yoshiyuki TAKAHASHI[3] and Kenichiro SHOHARA[3]
1) National Agriculture Research Center, 2) Faculty of Agriculture, University of Tokyo, 3) Japan Plant Protection Association

Research Period : 1978-91
Research Site : Tropical Agriculture Research Center and National Agriculture Research Center

Of the 10 insect-borne rice viruses identified in Asia, 7 multiply in insect vectors and are transmitted persistently throughout the life of the insects. Three of the persistent viruses are transovarially transmitted. Vector insects, such as planthoppers and leafhoppers, are widely distributed in Asia. Some of them are able to migrate over long distances and transmit viruses from one country to another in Asia, even across the sea. Therefore, the detection of viruses in viruliferous insects as well as in infected plants is important for analysing the virus epidemiology and developing control measures for the virus diseases of rice.

For the detection of the viruses, serological methods such as enzyme-linked immunosorbent assay (ELISA), simplified ELISA, latex flocculation test (LF), and passive hemagglutination test (PHA) have been used in some rice viruses. However, comparative efficiencies and the limitations and/or advantages of these methods have not been determined for each virus. In this study, we compared these four methods for practical detection of the viruses in rice plants and viruliferous insects in terms of sensitivity, applicability, reliability and cost. Based on this analysis, a manual for the detection of each rice virus in infected rice plants and viruliferous insects was prepared.

Key words: ELISA, simplified ELISA, latex flocculation test, passive hemagglutination test

Materials and Methods

Viruses, insects and plants

Virus sources were maintained in rice plants at the National Agriculture Research Center, Tsukuba, Japan. Rice stripe virus (RSV) and rice black-streaked dwarf virus (RBSDV) were maintained by successive transfers using the smaller brown planthopper, *Laodelphax striatellus*, while rice ragged stunt virus (RRSV) and rice grassy stunt virus (RGSV) were maintained by using the brown planthopper, *Nilaparvata lugens*. The green rice leafhopper, *Nephotettix nigropictus*, was used for the transmission of the rice dwarf virus (RDV), rice gall dwarf virus

(RGDV) and rice transitory yellowing virus (RTYV). The semi-persistent viruses, rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) or rice waika virus (RWV), were transmitted using another green leafhopper species, *Nephotettix virescens*. All the inoculated rice plants, belonging to the variety of Taichung Native 1, were grown in an air-conditioned greenhouse (27±3℃). Infected leaf samples were stored in a freezer and then tested for virus detection. More than 50 insect vectors exposed or not exposed to each virus were assayed, respectively in one experiment.

ELISA and simplified ELISA

Antisera against RDV, RGDV, RBSDV, RRSV, RTBV, RTSV, RWV, RTYV and RGSV exhibited titers of 1:2,000, 1:2,560, 1:4,000, 1:1,024, 1:2,560, 1:320, 1:640, 1:800 and 1:1,600, respectively in the precipitin ring interface test. Monoclonal antibodies against RSV with a titer of 1:1,600 in the same test were also used. Double antibody-sandwich ELISA procedure was used as standard ELISA. Simplified ELISA was performed according to the method described by Takahashi et al. (1987). Plates were coated with respective γ-globulin at the same concentration as that used in the standard ELISA and stored at 4℃ until use. After washing with PBS-T, the respective conjugate was mixed with an appropriate amount of plant homogenate in each well to obtain the final concentration of the conjugate as in the case of the standard ELISA procedure. For the detection of insects carrying viruses, individuals were placed in the wells of a coated plate. The insect was smashed with a vinyl chloride rod (ϕ 6 mm), which was wiped with a paper towel and used for smashing the other ones. An aliquot of 200 μl conjugate at the same dilution as that used in the standard ELISA was added to each well. After incubation with the sample-conjugate mixture for 1-2 hr at 37℃, the plates were observed for the reaction.

LF and PHA

Latex suspension was sensitized with each antiserum following the procedure described by Omura et al. (1984). For PHA, formalinized sheep erythrocytes were sensitized with the antiserum of RDV, RTBV, RTYV, RSV or RGSV.

Results and Discussion

Detection of virus antigens in plants

Reciprocals of dilution end points for the positive reactions of viruses in rice plants in the four methods are shown in Table 1. In general, the detection of the viruses

Table 1. Reciprocal of dilution end points of positive reactions in four serological methods for the detection of rice viruses

Virus[a]	Serological method[b]			
	ELISA	Simplified ELISA	LF	PHA
RDV	128,000[b]	32,000	8,000	16,000
RGDV	5,120	1,280	40	nt
RBSDV	1,280	80	–	nt
RRSV	640	80	10	nt
RTBV	2,560	160	40	40
RTSV	2,560	160	80	nt
RWV	3,200	640	40	nt
RTYV	25,600	25,600	–	–
RSV	512,000	64,000	16,000	16,000
RGSV	64,000	32,000	8,000	16,000

[a] Rice plant infected with each virus was used as the antigen.

[b] – = Negative reaction, nt = not tested.

This Table was adapted from Takahashi et al. (1991) with permission.

with systemic infection (RDV, RSV, RTYV, RGSV) was easier than that of phloem-restricted viruses (RBSDV, RGDV, RRSV, RTBV, RTSV, RWV, RTYV). Most viruses tested were detected efficiently by ELISA and simplified ELISA. RDV, RGSV, and RSV were also detected efficiently by LF and PHA, while the other viruses were hardly or not detected by LF and PHA.

Detection efficiency was compared between fresh or frozen leaf samples. No significant difference was observed between the samples in the A_{410} values in ELISA and simplified ELISA, and also in the results obtained by LF and PHA. Use of roots is recommended for practical diagnosis of three plant reoviruses RGDV, RBSDV and RRSV, because the antigen concentrations appeared to be higher in the roots than in the leaves (data not shown) as reported earlier for RGDV and RRSV. The concentration of another plant reovirus RDV in rice leaves was higher than that in the roots.

Detection of virus antigens in insects

ELISA and simplified ELISA enabled to detect all the viruses in individual insect vectors in which the transmission is persistent. LF enabled to detect RDV, RGDV, RSV and RGSV in individual vectors, and PHA also enabled to detect RDV, RSV and RGSV. Viruses which were transmitted transovarially (RDV, RGDV, RSV) were detected not only in individual adult insects but also in nymphs immediately after hatching. None of the methods used enabled to detect the RWV, RTSV and RTBV antigens in the respective insect vectors.

Evaluation of serological methods

Table 2. Recommended methods for the detection of rice viruses under different conditions

(1) For the screening of a large number of samples

Virus	Rice plant				Insect vector			
	ELISA	Simplified ELISA	LF	PHA	ELISA	Simplified ELISA	LF	PHA
RDV	○[a]	◎	△	△	○	◎	△	○
RGDV	○	◎	△	−	○	◎	△	−
RBDV	○	○	−	−	○	◎	−	−
RRSV	◎	○	△	−	○	◎	−	−
RTBV	◎	○	△	△	−	−	−	−
RTSV	◎	○	△	−	−	−	−	−
RWV	◎	○	△	−	−	−	−	−
RTYV	○	◎	−	−	○	◎	−	−
RSV	○	◎	△	△	○	◎	△	△
RGSV	○	◎	△	△	○	◎	△	△

(2) For immediate detection in a few samples

Virus	Rice plant				Insect vector			
	ELISA	Simplified ELISA	LF	PHA	ELISA	Simplified ELISA	LF	PHA
RDV	△	○	○	◎	△	○	△	◎
RGDV	△	○	◎	−	△	○	◎	−
RBDV	○	◎	−	−	○	◎	−	−
RRSV	○	◎	○	−	○	◎	−	−
RTBV	○	○	◎	△	−	−	−	−
RTSV	○	○	◎	−	−	−	−	−
RWV	○	○	◎	−	−	−	−	−
RTYV	○	◎	−	−	○	◎	−	−
RSV	△	○	◎	○	△	○	◎	○
RGSV	△	○	◎	○	△	○	◎	○

[a] ◎ : Most suitable and recommended method under each condition.
○ : Better method.
△ : Available method.
− : Method which does not enable or makes it difficult to detect the virus.

In mass-inspection of viruliferous insects, sample preparation requires a considerable amount of time for testing in LF, PHA or ELISA. The smashing method where individual insects pushed with a vinyl chloride rod directly in a well provides an adequate extraction of virus antigen. The time required for simplified ELISA can be shortened in this way. By adopting the direct smashing method, simplified ELISA showed a comparative advantage over LF and PHA in its simplicity in the detection procedure. Generally, the order of virus detection sensitivity was; ELISA ≧simplified ELISA≧LF≧PHA. On the other hand, the order of simplicity of the tests was; LF≧

PHA ≧ simplified ELISA ≧ ELISA.

Based on the results described above, the methods recommended for the practical detection of rice viruses are summarized in Table 2. In mass-indexing involving the monitoring of virus-carrying vectors for disease forecasting, and screening of rice cultivars for resistance to virus, sensitive and reliable methods should be developed for effective detection of virus antigens. ELISA and especially simplified ELISA are suitable for these purposes (Table 2). For immediate testing of a small number of plant samples with distinct symptoms, the use of LF and /or PHA is more practical and economical than that of ELISA.

The users can currently select serological methods depending on their objective, sample number, laboratory facilities available, and availability of skilled manpower. If coating γ-globulin and conjugate for ELISA, sensitized latex for LF, or sensitized sheep erythrocytes for PHA are available, the assays could be used more widely in the Asian countries. The development of practical methods of serodiagnosis of rice viruses will undoubtedly be suitable for the promotion of an integrated approach to the control of virus diseases of rice.

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