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First Report of Tomato Mosaic Tobamovirus from Malaysia

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Keywords: tomato mosaic tobamovirus, ToMV, *Lycopersicon esculentum*

ABSTRAK

Satu penyakit mosaik di tomato telah dilihat di Cameron Highlands dan menunjukkan sejenis tobamovirus adalah penyebab berasaskan kepada morfologi zarah virus dan pengeluaran simptom di Lycopersicon esculentum *Mill. Virus telah dikenalpasti sebagai 'tomato mosaic tobamovirus' (ToMV) melalui ciri-ciri julat perumah dan serologi.*

ABSTRACT

A mosaic disease of tomato was observed in Cameron Highlands, Malaysia and a tobamovirus was implicated as the cause based on virus particle morphology and reproduction of symptoms in Lycopersicon esculentum *Mill. The virus was identified as tomato mosaic tobamovirus (ToMV) based on host range and serological properties.*

INTRODUCTION

Tomato has been grown on a considerable scale in scattered 1 – 2 hectare plots for more than twenty years in Cameron Highlands, Malaysia, a cool, highland area 1500 m above sea-level. In 1987, tomato plants showing virus-like symptoms including mild foliar mottling were observed for the first time. Virus infection of tomatoes had not previously been reported in Malaysia. Examination of leaf dip preparations showed that the tomatoes were infected with rigid rod-shaped particles. In this paper we present evidence that the virus is an isolate of tomato mosaic tobamovirus (ToMV).

MATERIALS AND METHODS

Virus Isolates and Maintenance

Tomato mosaic tobamovirus, Dahlemense strain ATCC PV394 (ToMV-394) and tobacco mosaic tobamovirus, common strain ATCC PV135 (TMV-135), were purchased from the American Type Culture Collection (ATCC, Rockville, MD). ToMV-394 was propagated in tomatoes, and TMV-135 in tobacco (*Nicotiana tabacum* cv. Speight G28).

Inoculation and Host Range

The virus from leaves of field-infected tomato cv. Local 828 showing mottling symptoms was mechanically transmitted to *Nicotiana glutinosa* L. Tomato leaves were macerated in a mortar with 10 mM phosphate buffer (pH 7.0) and rubbed on to 600-mesh carborundum-dusted leaves of test plants grown in a temperature-controlled room supplemented with cool white lights at 25°C. Limited host range studies were carried out with inoculations made using infected tomato or tobacco leaves as the inoculum source. Indicator plants inoculated included *Lycopersicon esculentum* Mill. cvs. Local 828, Grosse Lisse, *Nicotiana tabacum* L. cvs. Speight G28, Xanthi,

Kentucky 15, Burley 49, *N. rustica* L., *N. megalosiphon* Arg., *N. benthamiana* Domin., *N. glutinosa* × *N. clevelandii*, *N. occidentalis* Wheeler, *Datura stramonium* L., *Gomphrena globosa* L. and *Chenopodium amaranticolor* Coste & Reyn. Similar inoculation tests also were carried out with ToMV-394 and TMV-135.

Electron Microscopy

Leaf-dip preparations for electron microscopy were prepared from infected tomato foliage. Small pieces of tomato leaf material were crushed in drops of 2% phosphotungstic acid, pH 6.8. A small drop of the extract was then placed on carbon-strengthened, Formvar-coated 400-mesh copper grids and viewed in the Philips HMG 400 transmission electron microscope.

Purification

The viruses from tomato, ToMV-394 and TMV-135, were purified from leaves of tomato *L. esculentum* cv. Grosse Lisse or tobacco *N. tabacum* cv. Speight G28 by the polyethylene-glycol (PEG) precipitation method of Hollings and Huttinga (1976) with modifications. The infected leaves were homogenised in 50 mM phosphate buffer (pH 7.5) containing 125 mM Na_2SO_3 (2 ml buffer/g tissue). Chloroform was added (1 ml/g tissue) and mixed well. After clarification, polyethylene glycol (PEG 6,000) was added to the supernatant (4 g PEG/100ml supernatant). The precipitate was collected, resuspended, and followed by one cycle of differential centrifugation. The viruses were further purified on a 10-40% sucrose density gradient. The virus fraction, recovered from density gradients with a fractionator, was sedimented and resuspended in the same buffer.

Serology

Polyclonal antisera to ToMV from Malaysia, ToMV-394 and TMV-135, were produced in rabbits immunized using a series of two intravenous, one subcutaneous and one booster injection. For each rabbit a first injection of 250 µg/ml antigen was administered followed by a second injection of 500 µg/ml intravenously after one week. Four weeks later, 1 mg/ml of antigen emulsified with an equal volume of Freund's complete adjuvant was injected subcutaneously. Booster injections were given subcutaneously with 450 µg/ml of antigen emulsified with Freund's incomplete adjuvant one month later. The rabbits were bled at weekly intervals starting one week after the last injection. Antisera with a minimum reciprocal titer of 64 by gel immunodiffusion tests were used in the serological studies.

The direct double antibody-sandwich (DAS) protocol of Clark and Adams (1977) and the indirect ELISA protocol of Lommel *et al.* (1982) were used to compare the viruses. The immunoglobulin (IgG) was purified from antisera by ammonium sulphate precipitation and chromatographed on DEAE 52 cellulose (Whatman Biosystems Ltd.) on a 1 × 8-cm Bio-Rad Econolumn. For DAS-ELISA, the microtitration plates (polystyrene, flat-bottom) were coated with IgG (1 µg/ml), followed by purified virus samples at two-fold dilutions and incubated at 4°C overnight, and alkaline phosphatase-conjugated IgG applied and incubated at 37°C for 2 h.

For the indirect-ELISA similar microtitre plates were used and coated with purified virus samples at two-fold dilutions. After washing, antiviral antibody at 10 µg/ml was added and incubated for 3 h. Goat anti-rabbit IgG conjugate was applied and incubated for 2 h. The washing procedures were carried out using the Titertek Microplate Washer (Flow Laboratories Inc., Australia) and the absorbance values were measured with a Titertek Multiskan Plus

Reader (Flow Laboratories Inc., Australia) at 405 nm 30-60 min after the enzyme substrate was added.

Immunoelectron microscopy was performed as described by Milne and Luisoni (1977) and Hill (1984). Purified ToMV from Malaysia, ToMV-394 and TMV-135, at a concentration of 200 µg/ml with ToMV from Malaysia antiserum diluted at 1/100 with 10 mM phosphate buffer (pH 7.0) and incubated in a humid chamber at room temperature for 15 min. The grids were then examined in the transmission electron microscope for particle decoration.

RESULTS AND DISCUSSION

Host Reactions

The following host plants produced necrotic local lesions with the virus isolated from tomato: *N. glutinosa, N. tabacum* cvs. Xanthi, Speight G 28, Kentucky 15, *N. rustica, N. occidentalis, N. megalosiphon, N. glutinosa* × *N. clevelandii, D. stramonium, C. amaranticolor* and *G. globosa*. Similar reactions were observed when ToMV-394 was inoculated to the indicator plant species. Symptoms in *N. glutinosa, C. amaranticolor, N. rnegalosiphon, N. rustica, N. tabacum* cvs. Xanthi and Kentucky 15 showed similarity to that of TMV (Zaitlin and Israel 1975; Brunt 1986). However, ToMV could be further differentiated from TMV by its nonsystemic reaction in *D. stramonium, N. rustica* (Brunt 1986) *N. tabacum* cv. Kentucky 15, *N. megalosiphon* and systemic latent infection in *N. tabacum* cv Burley 49. Mild systemic mottling symptom was observed in *L. esculentum* cvs. Local 828, Grosse Lisse. The symptom in tomato cv. Local 828 was similar to the symptom observed in the field (*Fig. 1*).

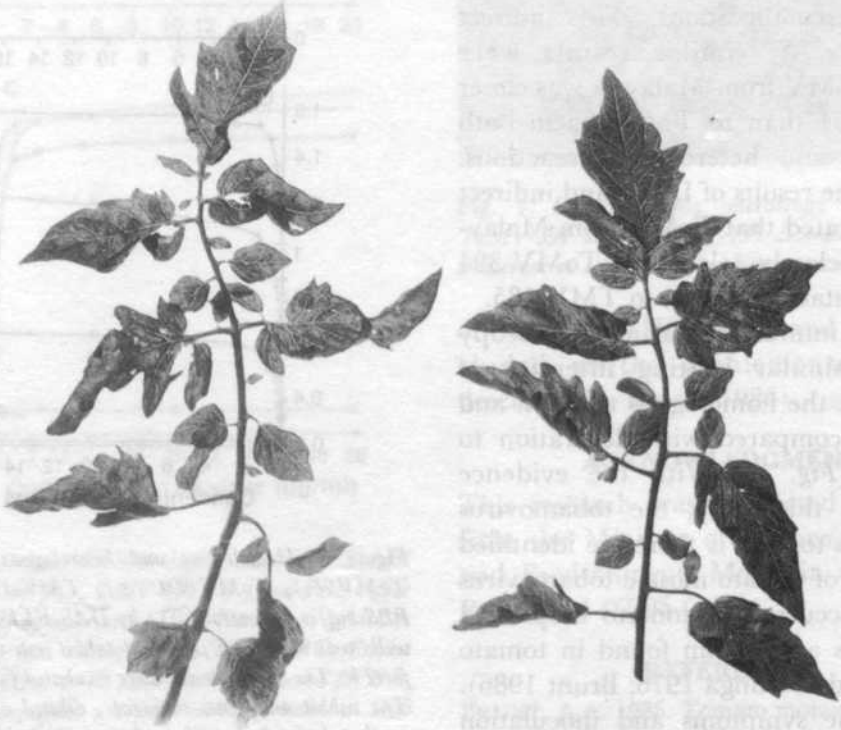

Fig 1: Field-infected (left) and inoculated (right) tomato with ToMV

Electron Microscopy

Field-infected tomato plants with mild mottling symptoms (*Fig. 1*) contained short rigid rod-shaped, virus-like particles. No virus particles were detected in symptomless plants. One UV-absorbing peak was observed on sucrose density gradients. Electron microscopic examination of the peak showed the same short, rigid, rod-shaped particles as those seen in leaf-dips. The modal particle length was 290 nm in purified preparations.

Serological Tests

When ToMV from Malaysia was allowed to react in both homologous and heterologous combinations in DAS-ELISA, it was clearly differentiated from ToMV-394 and TMV-135 (*Fig. 2*). The maximum reading was obtained in the homologous reaction. Similar reactions were also observed when ToMV-394 and TMV-135 antibodies were used in both homologous and reciprocal heterologous combinations. With indirect ELISA (*Fig. 3*) similar results were obtained; ToMV from Malaysia was closer to ToMV-394 than to TMV-135 in both homologous and heterologous reactions. Therefore, the results of DAS– and indirect ELISA indicated that ToMV from Malaysia is more closely related to ToMV-394 and more distantly related to TMV-135.

In the immunoelectron microscopy there was similar coating intensity of antibodies in the homologous reaction and ToMV-394 compared with decoration to TMV-135 (*Fig. 4*). With the evidence presented in this study, the tobamovirus isolated from tomato is therefore identified as an isolate of tomato mosaic tobamovirus because it occurred in tomato crops and TMV strains are seldom found in tomato (Hollings and Huttinga 1976; Brunt 1986). Based on the symptoms and inoculation studies in tomato cv. Local 828, the virus is the cause of the foliar symptoms in tomato

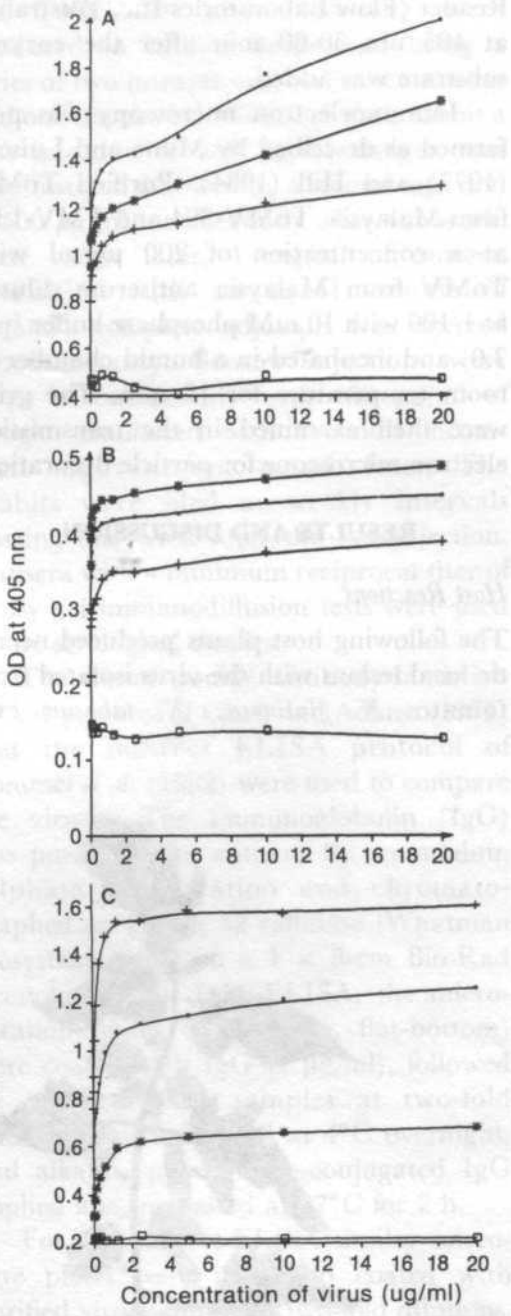

Figure 2: Homologous and heterologous reactions of ToMV (.), ToMV-394 (*), TMV-135 (+) and PBS buffer as control (□) by DAS-ELISA. Coating of wells was done with µg/ml of rabbit anti-virus globulins for 2 h. The viruses tested were incubated for 18 h at 4°C. The rabbit anti-virus conjugate, diluted at 1:3200 was incubated for 2 h. The substrate hydrolysis was 1 h. Antisera prepared against (A) ToMV, (B) ToMV-394 and (C) TMV-135.

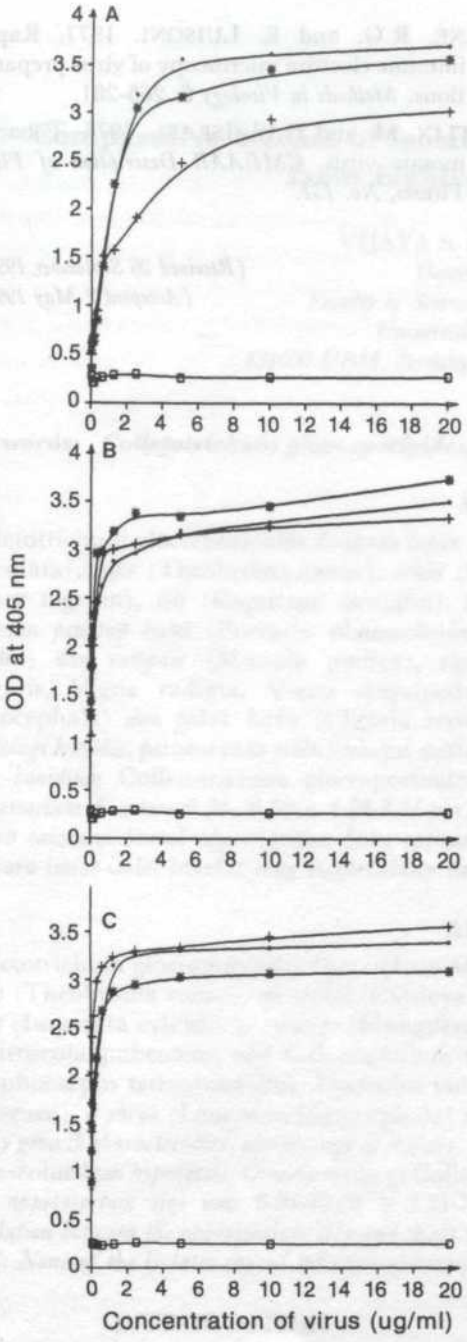

Fig. 3: Homologous and heterologous reaction of ToMV (.), ToMV-394 (*), TMV-135 (+) and PBS buffer as control (□) by indirect ELISA. The viruses diluted in coating buffer was incubated for 18 h at 4°C. Rabbit antivirus globulins at 10 µg/ml were incubated for 3 h, followed by conjugated goat anti-rabbit globulins at 1:00 dilution for 2 h. Substrate hydrolysis was 1 h. Antisera prepped against (A) ToMV, (B) ToMV-394 and (C) TMV- 135.

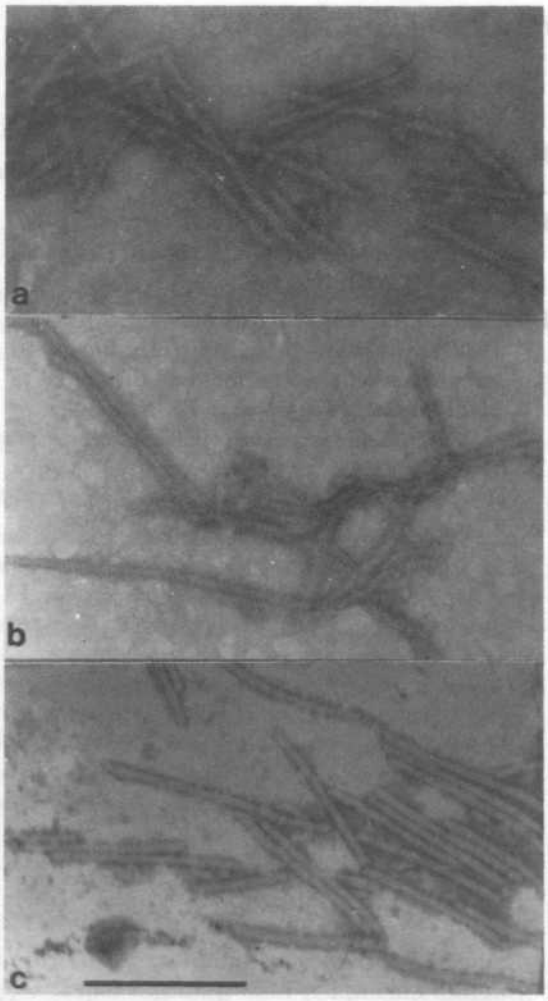

Fig. 4: Immunoelectron microscopy a. ToMV, b. ToMV-394 and c. TMV-135 decorated with ToMV antiserum at 1/100 dilution. Bar represents 300 nm.

on Cameron Highlands and can be included as the Dahlemense strain as described by Brunt (1986).

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Comparative Studies of Isolates of *Colletotrichum gloeosporioides* from Eighteen Malaysian Hosts

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Keywords: ***Colletotrichum gloeosporioides*, Malaysian hosts, anthracnose, pathogenicity**

ABSTRAK

Colletotrichum gloeosporioides *daripada lapan belas perumah di Malaysia yang terdiri daripada oren* (Citrus reticulata), *koko* (Theobroma cacao), *orkid* (Cattleya *sp.*), *rumput* (Imperata cylindrica), *lada hitam* (Piper nigrum), *cili* (Capsicum annuμm), *mango* (Mangifera indica) *dan kekacang* (legum) *iaitu tanaman penutup bumi* (Pueraria phaeseoloides, Centrosema pubescens *dan* Calopogonium mucunoides) *dan rumpair* (Mimosa pudica), *sayur-sayuran* (Psophocarpus tetragonolobus, Phaseolus vulgaris, Vigna radiata, Vigna sesquipedalis *dan* Arachis hypogaea), *pokok renik* (Leucaena leucocephala) *dan pokok herba* (Clitoria ternatea), *telah dikaji dari segi ciri-ciri pertumbuhan koloni, morfologi konidia, pertumbuhan pada berbagai media, suhu dan pathogenisiti ke atas hipokotil kacang* Phaeseolus. *Saiz konidium* Colletotrichum gloeosporioides *berada di antara 14.25-19.0 × 2.7-5.03 μm. Saiz-saiz appressorium di antara 6.34-10.08 × 5.28-7.31 μm dan ianya berbentuk globus/subglobus/lobus. Tiada korelasi di antara saiz dan bentuk appressorium. Suhu optimum untuk pertumbuhan adalah 28 dan 30°C. Tiada satu pun diantara isolat-isolat tersebut yang menyebabkan simptom-simptom infeksi pada hipokotil kacang* Phaseolus.

ABSTRACT

Colletotrichum gloeosporioides *from eighteen Malaysian hosts, namely mandarin orange* (Citrus reticulata), *cacao* (Theobroma cacao), *an orchid* (Cattleya *sp.*), *pepper* (Piper nigrum), *chilli* (Capsicum annum), *grass* (Imperata cylindrica), *mango* (Mangifera indica) *and legume cover crops* (Pueraria phaeseoloides, Centrosema pubescens, *and* Calopogonium mucunoides) *and a weed* (Mimosa pudica), *vegetables* (Psophocarpus tetragonolobus, Phaseolus vulgaris, Vigna radiata, Vigna sesquipedalis *and* Arachis hypogaea), *a shrub* (Leucaena leucocephala) *and a herbaceous vine* (Clitoria ternatea) *were examined for colony growth characteristics, morphology of conidia, growth on various media and temperatures and pathogenicity on* Phaseolus *bean hypocotyls. Conidium size of* Colletotrichum gloeosporioides *was 14.25-19.0 × 2.7-5.03 μm. The appressorium size was 6.34-10.08 × 5.28-7.31 μm and the shape was globose/sub-globose/lobed. No correlation between the appressorium size and shape was noticed. The optimum temperature for growth was 28 and 30°C. None of the isolates caused infection symptoms on* Phaseolus *bean hypocotyls.*

INTRODUCTION

Colletotrichum gloeosporioides (Penz.) Penz. & Saccs. causes anthracnose disease of flowers, fruits and leaves of various plants, causing serious postharvest damage of many tropical fruits such as mango, citrus, avocado, papaya, crops like cacao (Mordue 1971; Sutton 1980) and legumes such as *Stylosanthes* (Irwin and Cameroon 1978; Davis *et al.* 1992). The taxonomy of *C. gloeosporioides*, according to Sutton (1980), is based mainly on conidial morphology, which is extremely variable. Many authors have proposed groupings of *C. gloeosporioides* from tropical fruit crops (Hodson *et al.* 1993) and of *Stylosanthes* spp. in Australia (Dale *et al.* 1988; Braithwaite *et al.* 1990; Davis *et al.* 1992). These workers have used

conidial morphology, colony characters, disease symptoms, double stranded RNA and ribosomal and mitochondrial DNA polymorphisms to characterize the variablity among *C. gloeosporioides* isolates.

In Malaysia, tropical fruits are many and varied, while many legumes exist wild as well as cultivated and are used as vegetables, cover crops and as ornamental plants. *C. gloeosporioides* is present on the stems, leaves, flowers and fruits of many plants.

This present study deals with the *in vitro* examination of *C. gloeosporioides* to assess the extent of morphological and cultural variation within isolates of 18 Malaysian hosts.

MATERIALS AND METHODS

Isolates of *Colletotrichum gloeosporioides* were obtained from mandarin orange (*Citrus reticulata*), cacao (*Theobroma cacao*), an orchid (*Cattleya* sp.), pepper (*Piper nigrum*), chilli (Capsicum annuum), lallang grass (*Imperata cylindrica*), mango (*Mangifera indica*) and legumes: cover crops (*Pueraria phaeseoloides, Centrosema pubescens* and *Calopogonium mucunoides*), the weed *Mimosa pudica*, vegetables (*Psophocarpus tetragonolobus, Phaseolus vulgaris, Vigna radiata, Vigna sesquipedalis, Arachis hypogaea*), the shrub (*Leucaena leucocephala*) and the herbaceous vine (*Clitoria ternatea*). As almost all isolates of individual hosts had a similar appearance, only one isolate of each host was chosen for this study. Single spore colonies of each isolate were maintained on potato dextrose agar (PDA OXOID) until required.

Colony Characteristics and Growth Rates

The 18 isolates were grown on potato dextrose agar (PDA) at 28°C. A disc, 5 mm in diameter, of the fungal mycelium of each isolate was taken from the growing edge of 5-day-old colonies and transferred to the centre of PDA in 9-cm plastic petri dishes. Two replicate plates were made for each isolate. The plates were incubated for 5 days at 28°C. The diameter of the resulting colony was measured each day and the growth rate for each isolate was calculated. The colony characteristics were described using colony features such as mycelium, reproductive structures and colony appearances (modified from Davies *et al.* 1992).

Conidial and Appressorial Morphology

The isolates were grown on glucose caesamino acid medium at 28°C for 5 days under light. A spore suspension was prepared by the addition of 10 ml of sterile distilled water and agitating the colony surface. The spore suspension was filtered through muslin cloth, centrifuged at 1000 g for 4 min twice; the resultant pellet was resuspended in sterile distilled water to obtain a final concentration of 1×10^4 conidia per ml of water. The suspension was then (a) examined microscopically and the length and the width of at least 50 conidia per isolate were measured, (b) drops of conidial suspension, 10 µl in volume for each isolate, were placed on welled slides and incubated under moist conditions at 25°C for 12 h. The shape and size of the appressoria produced by the germinated conidia were recorded, and (c) slides from (b) were then flooded with 0.02% (w/v) aq. Calcofluor White M2R for 3 min, rinsed once with water and then examined under epifluorescence microscopy. The presence or absence of septa on the germinated conidia was noted.

Growth Studies

The effects of media and temperature on the growth of the colony of the different isolates were tested.

Media Effect on Colony Growth

The solid media used in the experiment

were potato dextrose agar (PDA), malt agar (MA), lima bean agar (LBA), oatmeal agar (OMA) and Czapek Dox agar (CDA-Difco). Two replicate plates of each colony for each isolate were prepared. A mycelial plug, 5 mm in diameter, was cut from a 7-day-old colony of each isolate and plated on freshly prepared agar plates of each of the media. Two radial diameters of the colonies were measured at right angles to one another after incubation at 28°C for 5 days.

Temperature Effect on Colony Growth

The temperatures studied were 15, 20, 25, 28, 30 and 35°C. Mycelial plugs 5 mm in diameter were transferred to freshly prepared PDA plates and the plates incubated at the above temperatures. The colony diameters of growth at two right angles were measured after 5 days. Two replicate plates were used for each isolate, and the experiment was repeated three times. Graphs were drawn using the average measurement of growth at each temperature, and the optimum temperature required for growth of each of the fungal isolates was determined from the graphs.

Pathogenicity Tests

The spore suspension 1×10^4 of each of the isolates was prepared after washing 3 times in deionised distilled water. Drops of 7 µl of these spore suspensions were used as inoculum on 5 points of a bean hypocotyl cut from 7-day-old seedlings of *Phaseolus vulgaris*. The ends of the hypocotyls were sealed in molten wax to prevent drying. Five hypocotyls were used for each isolate. Another 5 hypocotyls were inoculated with sterile deionised water and were used as controls. Prior to inoculation all hypocotyls were arranged on a rack and placed in plastic containers lined with moist tissue paper. The boxes were incubated under moist conditions at 25°C and the hypocotyls were examined periodically on Day 5, 7 and 14 after inoculation. The hypocotyls were first examined visually, then under a low-powered microscope to estimate the extent of lesion formation on the hypocotyl. An epidermal peel of the inoculated regions was also examined under a high-powered microscope and photomicrographs were taken.

RESULTS AND DISCUSSION

Colony characters, especially the appearance, colour and mycelial form, of the isolates of *C. gloeosporioides* from the 18 isolates varied greatly. The colour of *C. gloeosporioides* varied from white, to grey, to dark orange or pink-grey, while the reverse side of the colonies was of white, dark grey, orange or a mixture. Most colony margins were regular. Almost all isolates grew well on PDA with a growth rate of 11.00 – 15.91 mm per day except an isolate from *Cattleya* which had a growth rate of 7.00 mm per day (*Fig 1*). The mycelium was hyaline,

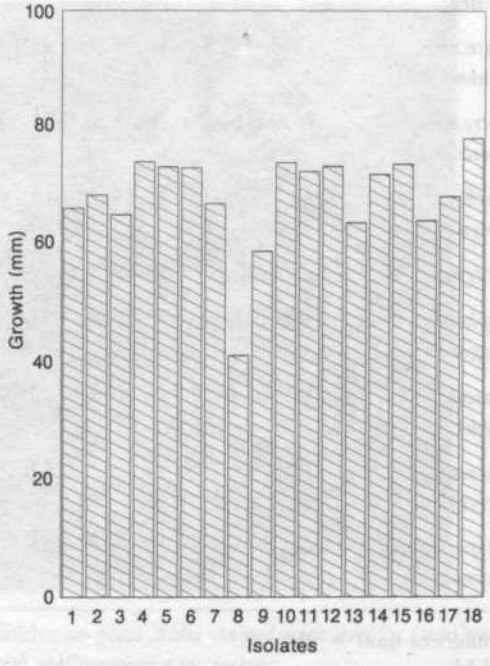

Fig. 1 Growth of C. gloeosporioides *on PDA at 28°C after 5 days*

TABLE 1
Size (μm) of conidia and appressoria of *Colletotrichum gloeosporioides* isolates

Isolates		Conidia		Appressorium		
Hosts	Code/ number	Length	Width	Length	Width	Shape**
Imperata cylindrica	Imper C/1	17.38 ± 0.44	4.99 ± 0.13	8.33 ± 0.43	7.18 ± 0.32	G
Citrus reticulata	CM/9	16.19 ± 0.24	4.09 ± 0.15	7.19 ± 0.26	5.40 ± 0.18	G/SG
Mimosa pudica	MPFI/2	17.10 ± 0.49	4.49 ± 0.13	9.19 ± 0.27	6.38 ± 0.27	G/SG
Vigna sesquipedalis	VSL2/18	17.35 ± 0.61	5.03 ± 0.11	7.80 ± 0.89	5.85 ± 0.17	G/SG
Pueraria phaseoloides	Peu B/10	15.69 ± 0.37	4.15 ± 0.25	9.08 ± 0.18	5.84 ± 0.15	G/SG
Piper nigrum	PipB003/11	14.31 ± 0.29	4.33 ± 0.13	6.55 ± 0.27	5.28 ± 0.17	SG
Vigna radiata	Khst/5	19.00 ± 1.19	4.31 ± 0.24	6.34 ± 0.25	5.33 ± 0.17	L
Theobroma cacao	CPO12/16	17.28 ± 1.23	4.68 ± 0.28	7.38 ± 0.15	5.38 ± 0.17	SG
Mangifera indica	M003/12	15.56 ± 0.44	4.48 ± 0.12	7.46 ± 0.27	6.34 ± 0.25	G
Clitoria ternatea	CIB010/13	14.93 ± 0.26	4.58 ± 0.11	10.08 ± 0.30	7.31 ± 0.11	SG
Capsicum annuum	Chi010/17	18.85 ± 0.35	2.70 ± 0.56	7.50 ± 0.20	6.20 ± 0.23	SG/L
Centrosema pubescens	Censt/14	16.81 ± 0.31	4.95 ± 0.05	9.90 ± 0.32	5.63 ± 0.14	SG/L
Arachis hypogaea	KtL/7	17.34 ± 0.15	4.54 ± 0.12	9.19 ± 0.20	6.56 ± 0.17	SG
Cattleya sp.	Cat CL/8	15.69 ± 0.52	4.00 ± 0.09	7.25 ± 0.14	6.15 ± 0.13	G/SG
Psophocarpus tetragonolobus	PT004/4	15.26 ± 0.27	4.59 ± 0.13	6.58 ± 0.08	5.85 ± 0.11	G/SG/L
Phaseolus vulgaris	FB002/6	16.99 ± 0.45	4.54 ± 0.09	6.70 ± 0.25	5.42 ± 0.11	G/SG/L
Leucaena leucocephala	PbL/15	14.25 ± 0.52	4.76 ± 0.10	8.24 ± 0.23	6.38 ± 0.24	SG/G
Calopogonium mucunoides	CAF/3	15.63 ± 0.94	4.88 ± 0.18	7.38 ± 0.33	5.50 ± 0.19	SG/G

Comfidence limit = 95%
* SEM
** G = Globose SG = Sub-globose L = Lobed

brown or both, sometimes abundant, at times sparse with either floccose, loose or compact growth. Some colonies formed sclerotia. Conidia formation was either on the hypha or in the acervulus, either only centrally or radially throughout the colony. The acervuli were either hyaline or dark, and at times a few dark setae were seen on the acervuli. Perithecia were formed by isolates from *Phaseolus vulgaris* and *Psophocarpus tetragonolobus*. The conidia were cylindric with obtuse ends or ovoid, the size varying from 14.25 – 19.0 µm × 2.7 – 5.03 µm (Table 1). The longest (19.0 µm) was produced by the *V. radiata* isolate and the shortest conidium (14.30 µm) by the isolates of *Piper nigrum* and *L. leucocephala*. All isolates produced conidia 4.0 – 5.03 µm in width, except for that of *C. annuum* with a width as low as 2.7 µm (Table 1). The spore measurements of the ovoid conidia of the above isolates fit within the measurements of spore sizes of *C. gloeosporioides* as given by Sutton (1980) and Mordue (1971). Giant conidia reported by Davis *et al.* (1992) were not found in this study of Malaysian isolates.

Germinated conidia of all isolates of *C. gloeosporioides* showed the presence of a septum which was very clearly seen after treatment with the fluorescent brightener, calcofluor (*Plate 1*). This character is said to be shown by all ovoid condia of *Colletotrichum* species except for *C. lindemuthianum* (O'Connell *et al.* 1992). Hence the isolates studied are definitely not *C. lindemuthianum* although the spores were cylidrical in shape and the majority were isolated from legumes.

The shape of the appressoria produced by the germinated conidia was variable, from globose (G), sub-globose (SG) to lobed (L). Size of the appressoria ranged from 6.34 – 10.08 µm long to 5.28 – 7.31 µm wide. Short, small appressoria (6.35 – 6.70 µm by 5.28 – 5.85 µm were produced by the isolates of *Phaseolus vulgaris, Psophocarpus tetragonolobus, V. radiata* and *Piper*

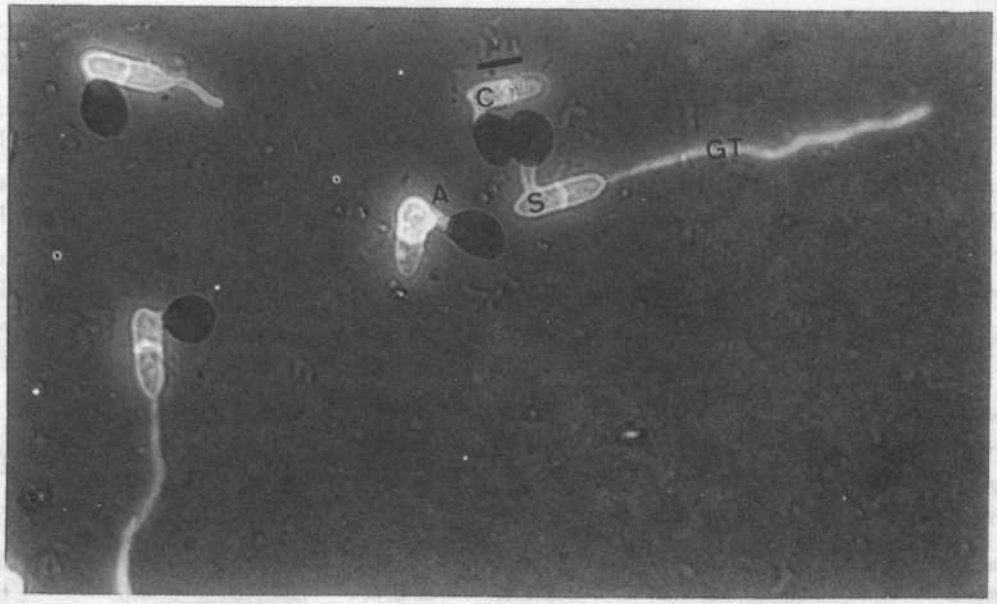

Plate 1. Germinating conidia of Colletotrichum gloeosporioides *on glass slides stained with 0.02% (w/v) aq. Calcofluor White M2R and viewed with epifluorescence microscopy*

A = appressorium, C = conidium, GT = germ tube, S = septum. Scale: 1cm = 10 µm

TABLE 2
Diameter of radial growth of *Colletotrichum gloeosporioides* isolates after five days

Isolates		Mean Diameter of Colony (mm)					
Hosts	Code/ number	MA	LBA	MEA	CDA	OMA	PDA
Imperata cylindrica	ImperC/1	61.00 ± 0.19*	53.25 ± 0.63	62.25 ± 0.63	66.50 ± 0.96	70.00 ± 1.41	66.25 ± 1.89
Citrus reticulata	CM/9	67.50 ± 2.06	69.25 ± 1.13	63.75 ± 0.85	59.75 ± 0.75	67.00 ± 1.58	58.88 ± 1.71
Mimosa pudica	MPF1/2	72.25 ± 1.32	71.00 ± 0.41	73.25 ± 0.75	65.25 ± 0.63	69.75 ± 2.87	68.50 ± 0.65
Vigna sesquipedalis	VSL2/18	80.75 ± 0.48	77.50 ± 1.19	69.50 ± 0.29	81.25 ± 2.66	81.50 ± 2.53	78.13 ± 0.32
Pueraria phaseoloides	Peu B/10	75.00 ± 2.04	81.75 ± 0.25	74.25 ± 0.25	70.25 ± 0.75	74.50 ± 0.29	74.75 ± 2.15
Piper nigrum	PipiB003/11	71.25 ± 1.03	81.50 ± 2.53	74.00 ± 0.91	69.25 ± 1.03	73.00 ± 1.16	72.50 ± 1.31
Vigna radiata	Khst/5	74.25 ± 1.12	71.50 ± 0.29	74.75 ± 0.48	74.00 ± 0.58	69.50 ± 3.52	73.25 ± 0.88
Theobroma cacao	CPO12/16	69.50 ± 0.65	68.50 ± 0.29	64.50 ± 1.76	64.75 ± 1.93	73.50 ± 3.58	64.13 ± 0.89
Mangifera indica	M003/12	68.25 ± 3.04	74.75 ± 0.48	71.75 ± 0.85	69.50 ± 0.29	54.25 ± 1.03	73.38 ± 1.69
Clitoria ternatea	CLB010/13	64.00 ± 1.47	73.50 ± 0.29	72.75 ± 0.85	61.50 ± 0.96	69.25 ± 1.44	63.75 ± 0.52
Capsicum annuum	Chi010/17	78.75 ± 0.25	76.50 ± 1.50	79.25 ± 0.63	71.25 ± 1.11	74.50 ± 0.65	68.50 ± 1.30
Centrosema pubescens	Censt/14	71.25 ± 2.50	67.00 ± 0.41	68.25 ± 0.75	65.25 ± 0.85	70.25 ± 0.25	72.00 ± 1.14
Arachis hypogaea	KtL/7	63.75 ± 0.25	74.00 ± 0.41	70.75 ± 0.48	78.00 ± 0.41	62.25 ± 4.92	67.00 ± 1.62
Cattleya sp.	Cat CL/8	52.50 ± 1.04	53.25 ± 2.18	49.50 ± 0.29	69.50 ± 0.29	53.00 ± 0.41	41.13 ± 0.55
Psophocarpus tetragonolobus	PT004/4	74.00 ± 0.36	71.63 ± 0.24	58.98 ± 0.88	62.50 ± 0.65	65.00 ± 0.98	74.13 ± 1.20
Phaseolus vulgaris	FB002/6	74.75 ± 0.86	71.75 ± 0.48	58.00 ± 0.41	62.50 ± 0.29	65.25 ± 0.48	73.13 ± 0.88
Leucaena leucocephala	PBL/15	79.00 ± 0.41	77.00 ± 1.47	74.00 ± 0.41	69.25 ± 0.48	73.50 ± 0.65	73.00 ± 0.41
Calopogonium mucunoides	CAF/3	69.00 ± 0.41	71.25 ± 1.18	65.75 ± 0.25	60.25 ± 0.48	70.00 ± 0.00	65.13 ± 1.25

Confidence limits = 95% *SEM

nigrum, while the largest were produced by the isolate from *Clitoria* (Table 1). There was no correlation between the size and shape of the appressoria although this had been reported by Cox and Irwin (1988).

All isolates grew well on all media tested although there were a few significant differences (Table 2). The growth of the isolate from *Cattleya* was relatively lower on most media except CDA compared with the growth of all other isolates. All isolates from legumes showed very good growth after 5 days (> 60 mm), some attaining > 80 mm as shown by the isolate from *V. sesquipedalis* on MA, CDA, OMA, and from *Pueraria* on LBA. The isolate from *Piper nigrum* also reached over 80 mm diameter in 5 days. All media supported good growth of *C. gloeosporioides*, with the growth rate on PDA at 28°C of all isolates varying slightly between 11.00 to 15.91 mm per day except for the slow growing isolate from *Cattleya* with a growth rate of 7.00 mm per day (Table 2).

Two optimum temperatures for growth were obtained for the isolates under study: (i) the optimum temperature for the 9 isolates from *Capsicum annuum, Vigna radiata, V. sesquipedalis, Pueraria phaseoloides, Calopogonium muconoides, Centrosema pubescens, Psophocarpus tetragonolobus, Clitoria ternatea* and *Leucaena leucocephala* was 28°C for growth on PDA, (ii) for the isolates from *Cattleya, Citrus reticulata, Imperata cylindrica, T. cacao, Piper nigrum, Mangifera indica, Phaseolus vulgaris, Mimosa pudica* and *Arachis hypogaea* the optimum temperature was 30°C (*Fig. 2a, b*). In general, *C. gloeosporioides* grows well between temperatures of 25-30°C but at 15 and 35°C growth was reduced. The optimum temperatures for growth of *C. gloeosporioides* on Malaysian hosts agree

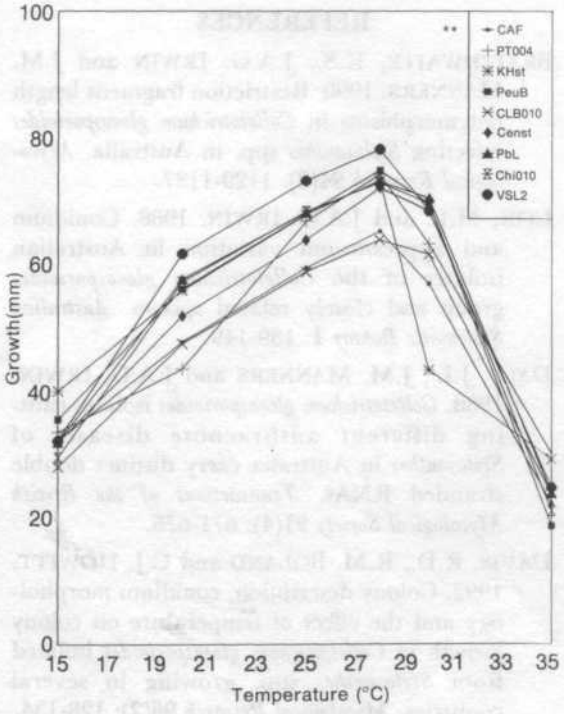

Fig. 2a. Temperature effect on the radial growth of Colletotrichum gloeosporioides *on PDA after 5 days*
**Isolates that showed optimum growth at 28°C

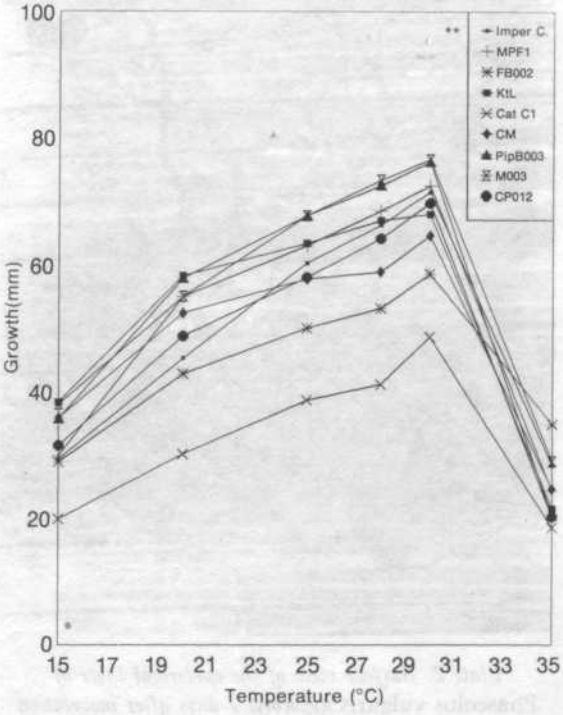

Fig. 2b. Temperature effect on the radial growth of Colletotrichum gloeosporioides *on PDA after 5 days*
**Isolates that showed optimum growth at 30°C.

with the results of previous workers (Cox and Irwin 1988; Davis *et al.* 1992).

All the bean hypocotyls inoculated with the various isolates of *C. gloeosporioides* showed slight brown discoloration as tiny brown spots over the epidermal cells of the inoculated regions. The intensity of the epidermal spots varied only slightly between the isolates. Examination of the epidermis showed germinated conidia and germ tubes ending with appressoria. Water-soaked regions appeared 5-7 days after inoculation and by Day 14 acervuli with pink glistening spore masses appeared over the entire hypocotyl length. Some acervuli had black setae. When viewed under the microscope the epidermal cells appeared to contain brown inclusions (*Plate 2*), indicating host reaction to infection. Although brown spots or dicolorations were seen, no further disease symptoms were observed. The subsequent water-soaked regions may be a consequence of weakened condition of the hypocotyls due to the incision on the hypocotyls or of ageing, which allows the mycelium to penetrate and colonize the soft, weakened plant tissues before forming the acervuli and conidia.

Plate 2. Surface view of the epidermal layer of Phaseolus vulgaris *hypocotyl 7 days after inoculation with conidia of* Colletotrichum gloeosporioides

A = appressorium, E = epidermal cell, EB = browned epidermal cell. Scale: 1cm = 10 μm

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Responses of Winged Bean (*Psophocarpus tetragonolobus*) to Mycorrhiza Inoculation in Pot and Field Trials

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ABSTRAK

Dua kajian di rumahijau dan satu kajian di ladang telah dijalankan untuk mendapat maklumat kemungkinan mengeksploit faedah kehadiran kulat mikoriza vesikul arbuskul dalam pertanian di Malaysia. Dalam kajian pertama, kacang kelisa (Psophocarpus tetragonolobus) *telah ditanam dalam tanah Serdang disucihama. Perkembangan endofit ini dinilai setiap dua minggu. Kajian kedua menggunakan tanah Serdang dan Munchong yang tidak disucihama, dengan 3 paras baja fosforus (P) dan diberi inokulasi dengan* Acaulospora laevis, Glomus macrocarpum, Glomus mosseae, *campuran spesies* Glomus *dan* Scutellospora calospora. *Kesemua inokula kecuali* S. calospora *meningkatkan pertumbuhan kacang kelisa dengan bererti, disamping meningkatkan kepekatan tisu N, P dan K.* Glomus mosseae *merupakan spesies yang superior. Dalam kajian di ladang,* Acaulospora laevis, Scutellospora calospora *dan* Glomus mosseae *telah diinokulatkan kepada kacang kelisa ditanam pada tanah yang tidak disucihama, dengan atau tanpa baja fosforus. Kehadiran* Glomus mosseae *mampu meningkatkan pembentukan bunga secara bererti ($P < 0.05$) (4.5 bunga/pokok), khususnya pada kadar baja P yang sederhana (60 kg P ha^{-1}).*

ABSTRACT

Two greenhouse experiments and one field trial were conducted to provide information on the possibility of exploiting the beneficial effects of vesicular-arbuscular mycorrhizal fungi in Malaysian agriculture. In the first study, winged bean (Psophocarpus tetragonolobus) *was grown in steam-sterilized Serdang soils and the development of the endophytes evaluated fortnightly. The second experiment was conducted in unsterilized Serdang and Munchong soils respectively with three levels of P and/or inoculated with* Acaulospora laevis, Glomus macrocarpum, Glomus mosseae, *a mixture of* Glomus *species and* Scutellospora calospora. *All inocula, except for* S. calospora, *significantly enhanced growth throughout the course of the experiments and increased N, P and K concentrations in the plant tissues.* G. mosseae *was superior to the rest. In the field trial,* Acaulospora laevis, Scutellospora calospora *and* Glomus mosseae *were inoculated into winged bean grown in unsterilized field soil, with or without phosphate fertilizer. Inoculation of winged bean with* G. mosseae *significantly ($P < 0.05$) increased inflorescence formation (4.5/plant), particularly at an intermediate level (60 kg ha^{-1}) of P fertilizer.*

INTRODUCTION

The beneficial effects of vesicular-arbuscular mycorrhizal (VAM) fungi on plant growth are well documented (Abbott and

Robson 1982; Harley and Smith 1983; Mosse 1986; Hall 1988). There is also a growing body of evidence that shows it may be possible to exploit the beneficial effects of VAM in agriculture (Daniels *et al.* 1981; Ganry *et al.* 1982; Abbott and Robson 1984). In developing countries, the relative cost of chemical fertilizer is high and consequently restricts their use. Therefore, there is considerable interest in the use of alternative fertilizers and the exploitation of symbionts such as rhizobia and VAM (Bagyaraj *et al.* 1979; Harris *et al.* 1985; Hall 1988; Azizah 1991; Kumaran and Azizah 1995). Reproducibility of results obtained from pot studies under controlled conditions is of prime importance if mycorrhiza inoculation is to be successfully introduced under uncontrolled conditions. The experiments described here were designed to screen a number of VAM fungi for effectiveness in enhancing growth of winged bean (*Psophocarpus tetragonolobus*) in Malaysian soils.

MATERIAL AND METHODS

Soils

Two greenhouse trials were established in unsterilized Serdang and Munchong soils (Table 1) and in Serdang soil, which had been steam-sterilized for 1 h at 100°C. The pH of the Serdang and Munchong soils was raised from the respective values of 4.6 and 4.4 to pH 6.0 by incorporating ground magnesium limestone (GML) at the rates of 2.3 and 2.7 g kg^{-1} soil respectively.

TABLE 1
Physicochemical properties of Serdang and Munchong soils

Physicochemical property	Serdang	Munchong
Horizon	Ap	Ap
+Depth (cm)	0 –15	0 – 15
+Coarse sand (%)	14.0	7.7
+Fine sand (%)	59.0	27.8
+Silt (%)	3.0	13.3
+Clay (%)	25.6	51.2
Organic C (%)	1.08	1.68
Total N (%)	0.11	0.13
Extractable P (µg/g)	6.31	2.97
pH (H_2O)	5.10	4.78

+ Paramananthan (Pers. comm.)

In Experiment 1, 1 kg sterilized Serdang soil was used to fill each 12-cm diameter pot and the following basal fertilizers were added: urea, at the equivalent rate of 14 kg N ha^{-1}, triplesuperphosphate (TSP), at 12 kg P ha^{-1} and muriate of potash (MOP), at 30 kg K ha^{-1}.

In Experiment 2, unsterilized Serdang and Munchong soils were used; 5 kg of each soil type in each 25-cm diameter pot lined with plastic. The following basal fertilizers were added: urea, at the equivalent rate of 14 kg N ha^{-1}, MOP at 60 kg K ha^{-1}, and three levels of TSP at 0, 30 and 60 kg P ha^{-1} respectively.

In Experiment 3, the field trial was conducted 4 months later in Experimental Plot No. 2 of Universiti Pertanian Malaysia, Serdang where the mean annual rainfall is 2000 mm and the mean annual air temperature is 24°C. The soil is an alluvium (old mining land) with pH(H_2O) 4.8 and 20µg bicarbonate extractable P. The site selected was overgrown with mixed weed species and had not received any form of fertilizer for the previous two years. After it had been ploughed three times in order to reduce soil heterogeneity, 2.3 t/ha GML was added to raise the pH to 6.0.

Endophytes

Five endophyte treatments were used in Experiment 1. They were: *Acaulospora laevis*, *Glomus macrocarpum*, *Glomus mosseae*, a mixture of *Glomus* species, and *Scutellospora calospora*. All species except *Glomus macrocarpum* were obtained from Dr. I.R. Hall of New Zealand. *G. macrocarpum* was supplied by Dr. K.R. Krishna of ICRI-

SAT, India. These species were propagated in the greenhouse of Universiti Pertanian Malaysia for a period of 9 months using *Setaria anceps* var. *splendida* as the host plant (Azizah and Omar 1987). All the VAM species above except *G. macrocarpum* were used in Experiment II, which was set up 1 month later.

Experiment 1: 50 g soil plus spores, mycelium and colonized root segments of *Setaria* were added to each inoculated pot. The inoculum was spread in a thin layer 5 cm below the level where the seeds were to be planted. Control plants were similarly treated using soil inocula grown with uninoculated *Setaria*. The plants were watered with distilled water twice daily for the first 14 days of growth and later as needed for the duration of the experiment.

Experiment 2: 100 g soil inoculum was used per pot; otherwise the procedure was identical to Experiment 1.

Experiment 3: 50 g soil inoculum containing either *A. laevis*, *G. mosseae*, *S. calospora* or sterilized soil was thinly spread 8 cm below each planting hole. In the *Rhizobium*-inoculated plots, a suspension of *a Rhizobium* culture was applied with a watering-can after the seeds were sown. Two pre-soaked seeds were sown per planting hole with 40 cm between plants and 45 cm between rows. Thinning was carried out one week after sowing. As the seedlings grew they were supported on string hung from a network of 1.5-m high wire trellises supported with wooden beams.

Experimental Design

The pots in each experiment were placed on wooden benches and randomized in blocks in the greenhouse.

Experiment 1: A 6 × 6 factorial combination consisting of the following treatments: five VAM fungal species plus one control, and six harvests. Only one plant was planted per pot, with each treatment replicated three times. Three plants from each treatment were harvested at fortnightly intervals.

Experiment 2: A randomized complete block design comprising the following treatments: four VAM fungal species (*Acaulospora laevis*, *Scutellospora calospora*, *Glomus mosseae* and mixed *Glomus* species, designated as Alae, Scal, Gmos and Gmix respectively in the text); two soil types (Serdang and Munchong), and three levels of P fertilizer – 0, 30 and 60 kg TSP ha^{-1} (designated as P_0, P_1 and P_2 respectively). There was also one plant per pot. with three replications per treatment. The mycorrhizal treatment (+R) was treated with a *Rhizobium* strain, RRIM 56; the control (–R) was not.

Experiment 3 (conducted four months later): The experimental design was a 3 × 5 factorial laid out in 4 randomized complete blocks giving a total of 60 plots. Each plot measured 2.6 × 2.7 m. The treatments included inoculation with: *A. laevis* + *Rhizobium*, *G. mosseae* + *Rhizobium*, *S. calospora* + *Rhizobium*, no inoculation with either VAM or *Rhizobium*, and inoculation with *Rhizobium* alone.

Harvests and Plant Analysis

Experiment 1: Successive harvests of three randomly selected replicates from each treatment were made 14 days after sowing, and continued thereafter at fortnightly intervals until 12 weeks after inoculation. At each harvest, fresh weights of root and shoots and also shoot dry weights were recorded. Randomly selected root samples were cleared of adhering debris, rinsed thoroughly and then stored in 10% formalin acetic acid (FAA). These root segments were then cleared in 10% KOH and stained with trypan blue (Phillips and Hayman 1970). A total of 90% 1mm-root sections per treatment was assessed. The

percentage of root length in the stained sample colonized by mycorrhizal fungi was recorded as positive and calculated using the following formula:

$$\% \text{ VAM colonization} = \frac{\text{No. of VAM positive segments}}{\text{Total no. of segments scored}} \times 100$$

Experiment 2: The plants were harvested 16 weeks after sowing. Plant parts were weighed immediately after harvest, and again after drying to constant weights at 72°C (approx. 2 days). The dried shoots were then ground. Twenty-five mg of the ground material was then wet ashed by digesting with 5 ml concentrated H_2SO_4 and oxidised with H_2O_2 (Thomas *et al.* 1967). The digest was subsequently made up to 250 ml, and N and P levels determined with a Technicon^R autoanalyser using the ascorbic acid method for P and the alkaline salicylate method for N. K concentrations were measured with a flame photometer.

Experiment 3: Fifty days after sowing, leaf numbers from 12 plants in each plot were recorded and after a further 20 days, numbers of fully opened flowers in each plot were counted. Untimely heavy rainfall produced over-luxuriant plant growth which broke the main trellis support and the experiment had to be terminated three weeks after the flower count was completed.

All the data obtained except percentage root colonization were subjected to analysis of variance (ANOVA) using GENSTAT. Percentage of root colonization was subjected to a chi-square test for test of significance.

RESULTS

Formation of Mycorrhizae

Good infection of winged bean roots as a result of inoculation with the five mycorrhizal species was observed (Experiment 1). However, positive signs of infection by these endophytes only became obvious 4 weeks after inoculation, after which infection of the roots increased with increase in sampling time (Table 2).

There was heavy infection (63%) of the roots inoculated with A. *laevis* from week 8 onwards. At age 4 weeks, development of mycorrhiza was greater in plants inoculated with *G. macrocarpum*, with 58% of the roots colonized. Plants inoculated with *G. mosseae* had 49% and 70% (with internal hyphae and arbuscules) mycorrhizal infection at 4 and 6 weeks after inoculation, respectively.

Results obtained from the chi-square test showed that differences in root colonization pwrcentage by the different VAM species were only significant at the first sampling period, i.e. 4 weeks after inocula-

TABLE 2
Percentage colonization of winged bean roots by five mycorrhizal fungi with time of harvest

	Sampling weeks				
	W4*	W6	W8	W10	W12
+*A. laevis*	38.0	53.5	63.0	66.3	70.2
+*G. mosseae*	49.0	70.0	73.3	74.5	78.8
G. macrocarphum	58.0	63.1	64.3	68.7	74.4
+Mixed *Glomus* spp.	34.0	52.2	26.7	69.4	75.1
+*S. calospora*	32.3	55.0	57.8	62.2	64.4

*For sampling week, Significant at $P < 0.05$
+For VAM species, Significant at $P < 0.05$

tion. Differences in root colonization percentage were not significant at 6, 8, 10 and 12 weeks of sampling due to the gradual increase in roots being colonized by all the VAM fungi.

Plant Growth

In Experiment 1, mycorrhiza, time of harvest (week) and interaction of these two factors significantly ($P < 0.05$) influenced growth and dry matter production of winged bean. Owing to heterogeneity in the sampling time variance data, each sampling time had to be analysed separately. However, applying a $\log_e$ transformation allowed all the data to be analysed together.

All five VA mycorrhizal fungal species significantly ($P < 0.001$) stimulated growth of winged bean compared to control plants. Shoot dry weights of mycorrhizal plants increased curvilinearly at all harvests except for plants treated with *S. calospora*. In these plants, shoot dry weights reached a maximum value of 2.7 g at week 10 and then declined to 2.4 g at the final harvest at week 12 (*Fig. 1*).

Fungal effectiveness was in the order: *A. laevis* = *G. mosseae* = mixed *Glomus* = *G. macro-carpum* >> *S. calospora* >> Control.

The root/shoot ratio values of mycorrhizal plants seemed to fluctuate with each harvest (*Fig. 2*). However, at the final harvest, all the mycorrhizal plants except for those treated with *S. calospora* had root/shoot ratios lower than the control plants. Both the *S. calospora*-treated and control plants showed a sharp increase in the root/shoot ratios from week 8 onwards.

In Experiment 2, soil, mycorrhiza and rate of phosphorus fertilization and interactions between these variables had significant ($P < 0.05$) effects on shoot (*Fig. 3*) and root dry weights (*Fig. 4*) in both Serdang and Munchong soils.

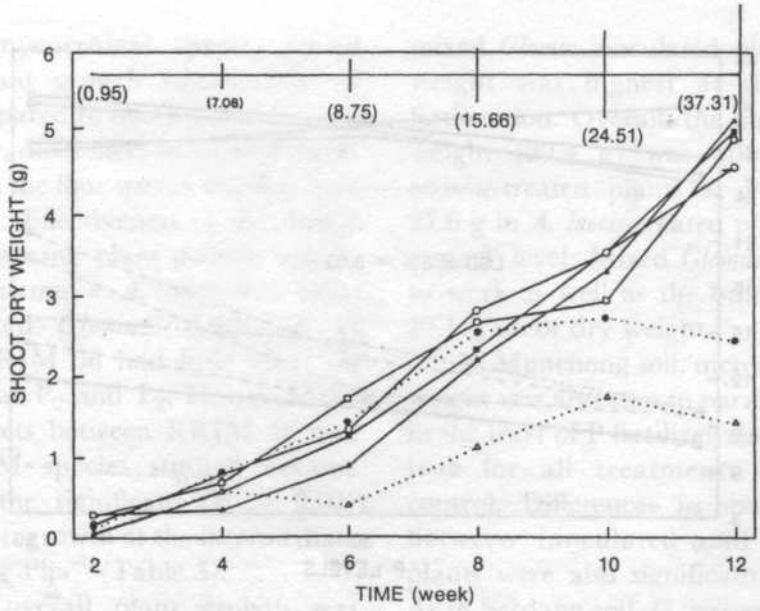


Fig 1. Shoot dry weight of Psophocarpus tetragonolobus *as affected by VAM inoculum and time of harvest*

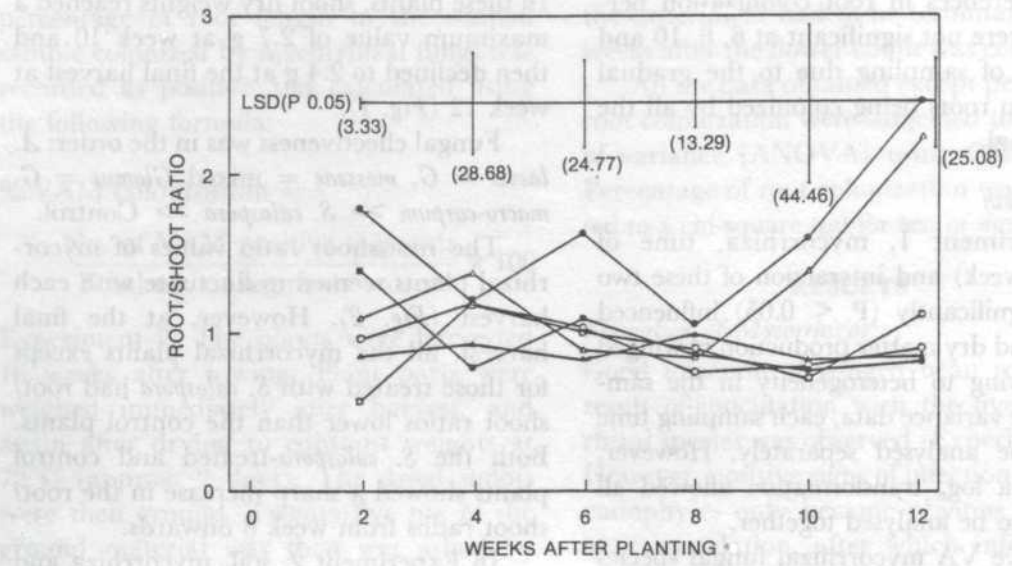


Fig 2. Root/shoot ratio of Psophocarpus tetragonolobus *as affected by VAM inoculum and time of havest*

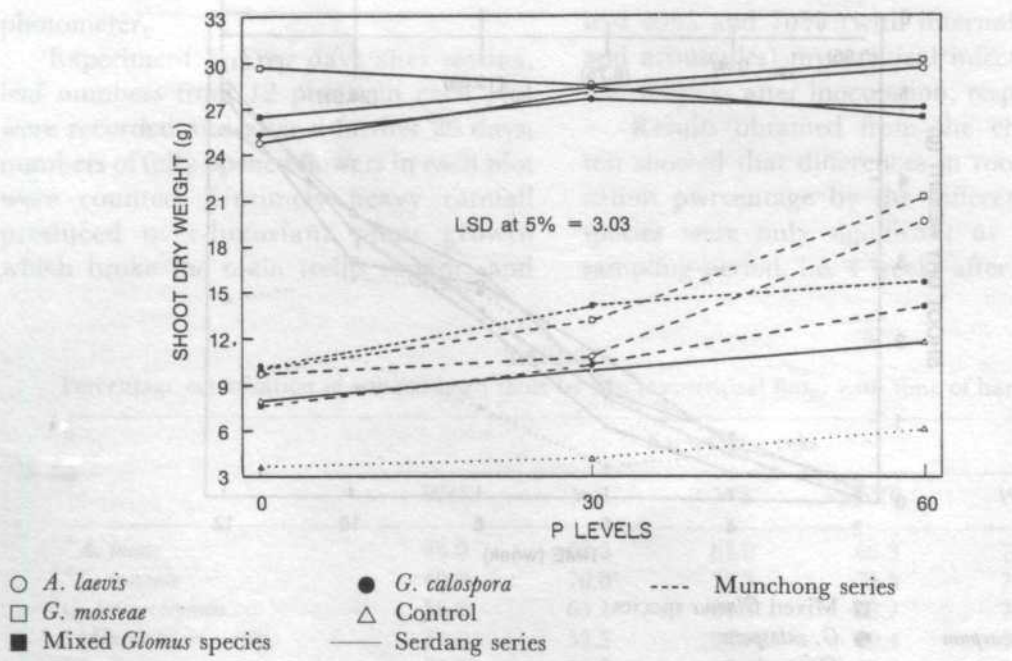


Fig 3. Shoot dry weight (g) of Psophocarpus tetragonolobus *as affected by VAM inoculum and P levels in Serdang and Munchong soils*

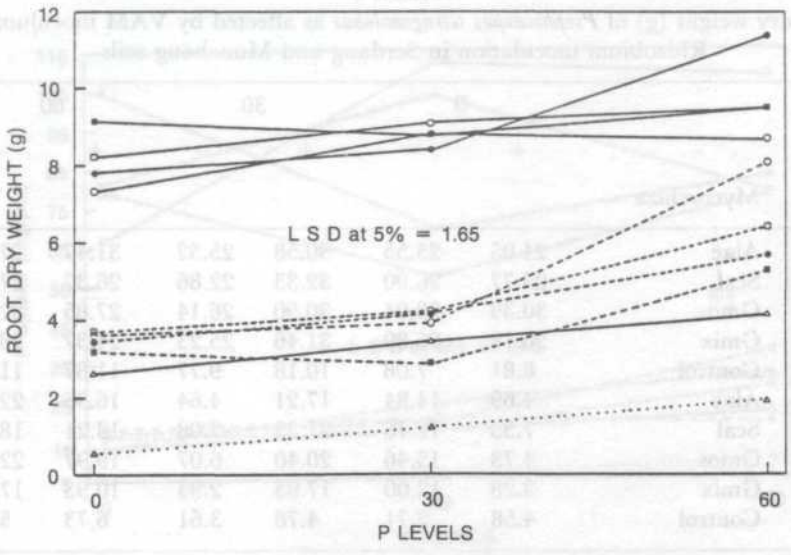


Fig 4. Root dry weights (g) of Psophocarpus tetragonolobus *as affected by VAM inoculum and P levels in Serdang and Munchong soils*

All the mycorrhizal species tested stimulated plant growth significantly ($P < 0.001$) compared to non-inoculated controls. However, differences in growth increments between the four species studied were not significant. Effectiveness of the fungal species in enhancing plant growth was in the order: *G. mosseae* > *A. laevis* = *S. calospora* > mixed *Glomus*. Addition of Rhizobium RRIM 56 had little effect on plant growth at P_0 and P_2. However, the synergistic effects between RRIM 56 and the four VAM species studied became evident with the significant ($P < 0.05$) increase in plant growth at the intermediate P level of 30 kg Pha^{-1} (Table 3).

The best overall plant growth was recorded in Serdang soil; shoot dry weights increased with increasing P level except for plants inoculated with *G. mosseae* and mixed *Glomus* species. In *G. mosseae*-treated plants, shoot dry weight decreased at P_1 while in mixed *Glomus*-inoculated plants, shoot dry weight was highest at this level of P fertilization. Overall, the highest shoot dry weight (29.4 g) was obtained from *G. mosseae*-treated plants at P_2, followed by 27.6 g in *A. laevis*-treated plants also at the same P level. Mixed *Glomus* also appeared to work as well as the other species, with 27.1 g shoot dry weight.

In Munchong soil, increase in shoot dry weight was also seen to parallel the increase in the level of P fertilizer added. This holds true for all treatments including the control. Differences in shoot dry weight between inoculated and uninoculated plants were also significant ($P < 0.005$). As in Serdang soil, *G. mosseae* and *A. laevis*-treated plants recorded the highest shoot dry weights of 14.8 g and 13.6 g respectively. Plant growth was stimulated less in Munchong than in Serdang soil.

Results obtained from the two pot trials

TABLE 3
Shoot dry weight (g) of *Psophocarpus tetragonolobus* as affected by VAM inoculum and Rhizobium inoculation in Serdang and Munchong soils

Phosphorus		0		30		60		
Rhizobium		+	−	+	−	+	−	
Soil	Mycorrhiza							
Serdang	Alae	24.05	25.55	30.58	25.52	31.47	27.67	
	Scal	23.77	26.90	32.33	22.86	26.37	27.67	
	Gmos	30.39	28.94	30.90	26.14	27.85	32.33	SED
	Gmix	26.18	26.89	31.46	25.23	24.37	28.33	2.138
	Control	8.81	7.08	10.18	9.77	11.87	11.65	
Munchong	Alae	4.69	14.84	17.21	4.64	16.96	22.34	
	Scal	7.55	12.76	21.33	7.08	13.21	18.12	
	Gmos	4.73	15.46	20.40	6.07	19.97	22.23	LSD5%
	Gmix	3.28	12.00	17.83	2.93	10.93	17.33	4.28
	Control	4.58	2.71	4.78	3.61	6.73	5.43	

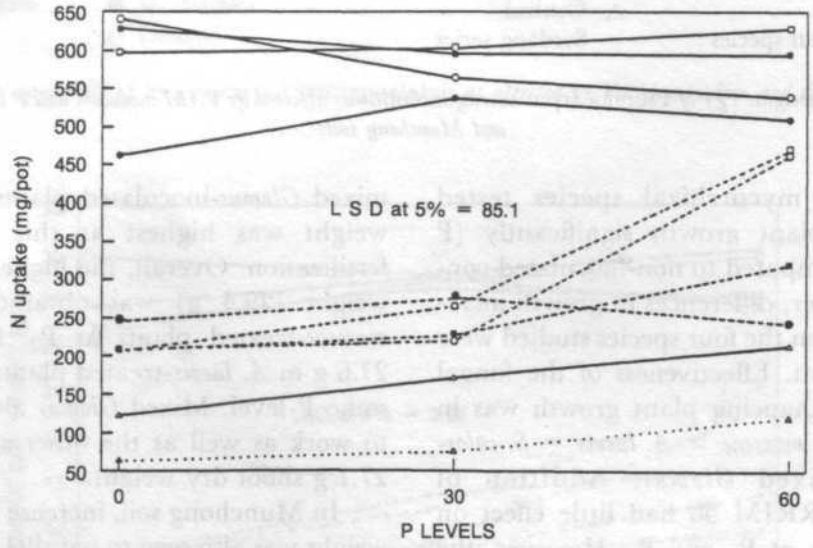
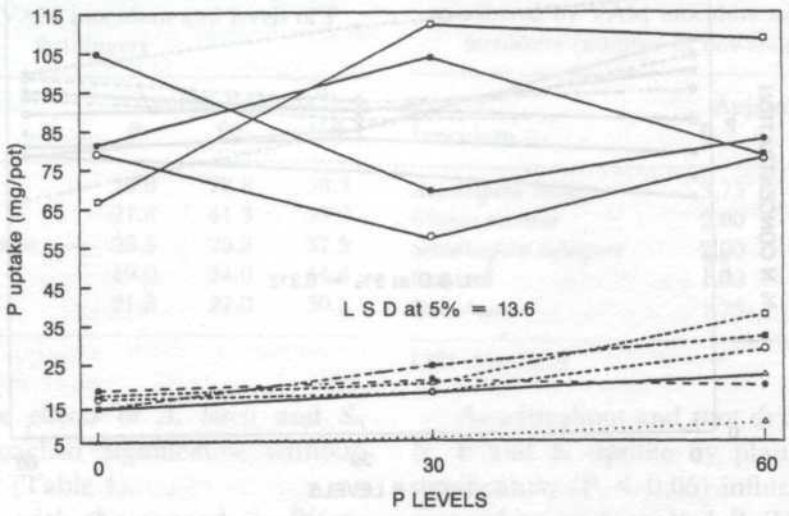


Fig 5. Uptake of N (mg/plant) by Psophocarpus tetragonolobus *as affected by VAM inoculum and P levels in Serdang and Munchong soils*

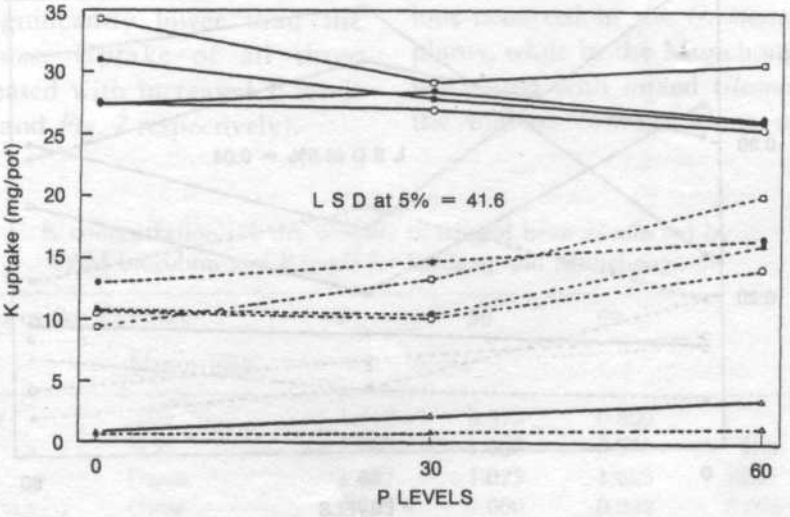
showed the same trends as those for leaf number and initiation of flowering in Experiment 3 (Table 4 and 5 respectively).

Overall, P had a significant ($P < 0.05$) effect on leaf number (Experiment 3). Analysis of the data using linear comparison of means showed that *G. mosseae* significantly stimulated growth, particularly at 60 kg P



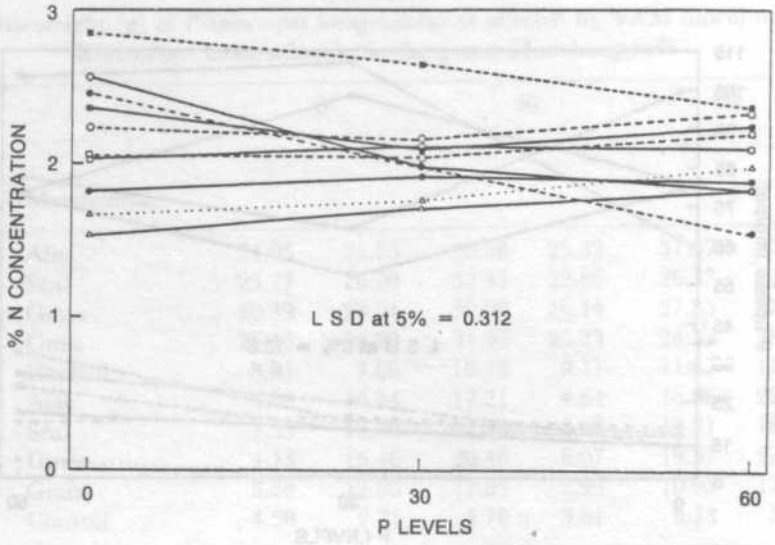
○ *A. laevis* ● *G. calospora* ---- Munchong series
□ *G. mosseae* △ Control
■ Mixed *Glomus* species —— Serdang series

Fig 6. Uptake of P (mg/plant) by Psophocarpus tetragonobus *as affected by VAM inoculum and P levels in Serdang and Munchong soils*



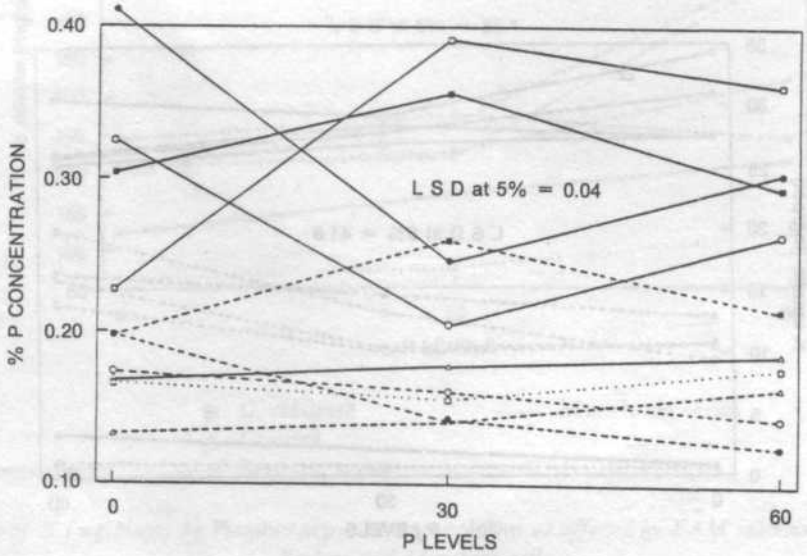
○ *A. laevis* ● *G. calospora* ---- Munchong series
□ *G. mosseae* △ Control
■ Mixed *Glomus* species —— Serdang series

Fig 7. Uptake of K (mg/plant) by Psophocarpus tetragonolobus *as affected by VAM inoculum and P levels in Serdang and Munchong soils*



○ *A. laevis* ● *G. calospora* ---- Munchong series
□ *G. mosseae* △ Control
■ Mixed *Glomus* species —— Serdang series

Fig 8. Percentage of N Concentrations in Psophocarpus tetragonolobus *shoots as affected by VAM inoculum and P levels in Serdang and Munchong soils*



○ *A. laevis* ● *G. calospora* ---- *Munchong series*
□ *G. mosseae* △ *Control*
■ *Mixed Glomus* species —— Serdang series

Fig 9. Percentage of P concentrations in Psophocarpus tetragonolobus *shoots as affected by VAM inoculum and P levels in Serdang and Munchong soils*

TABLE 4
Leaf number per plant of *Psophocarpus tetragonolobus* as affected by VAM inoculum and levels of P fertilingers

Inoculum	Applied P (kg ha^{-1}) 0	60	150
Acaulospora laevis	28.8	28.8	38.3
Glomus mosseae	21.3	41.3	39.0
Scutellospora calospora	28.5	25.8	37.5
Control	19.0	34.0	44.8
Rhizobium	21.8	27.0	30.5

LSD (5%): 13.02

TABLE 5
Initiation of flowering in *Psophocarpus tetragonolobus* as affected by VAM inoculum and levels of P fertilizers (number of flowers per plant)

Inoculum	Applied P (kg ha^{-1}) 0	60	150
Acaulospora laevis	3.75	0.75	1.21
Glomus mosseae	2.00	4.50	1.00
Scutellospora calospora	2.00	0.25	0.75
Control	1.00	2.50	2.00
Rhizobium	1.75	1.25	1.00

LSD (5%): 3.19

ha^{-1} whilst the effects of *A. laevis* and *S. calospora* approached significance without any P fertilizer (Table 4).

Compared with the control + *Rhizobium* treatment, the *G. mosseae* treatment significantly ($P < 0.05$) initiated earlier flowering in winged bean plants receiving 60 kg P ha^{-1} (Table 5). No other effects were significant.

N, P, K Uptake and Concentrations in Shoots

N, P and K uptake by the non-inoculated plants was significantly lower than the inoculated ones. Uptake of all three nutrients increased with increased P levels (*Fig. 5*, *Fig. 6* and *Fig. 7* respectively).

As with shoot and root dry weights, the N, P and K uptake by plant shoots was significantly ($P < 0.05$) influenced by soil, mycorrhiza and applied P. The uptake of all three elements was higher from Serdang than from Munchong soil.

Concentrations of N (*Fig. 8*), P (*Fig. 9*) and K (Table 6) in winged bean shoots were increased twofold through symbiosis with VAM in both plants grown at P_0 from Serdang and Munchong soils. In Serdang soil, the highest shoot P and K concentrat ions occurred in the *G. mosseae*-inoculated plants, while in the Munchong soils, plants inoculated with mixed *Glomus* species had the highest concentrations of P and K.

TABLE 6
K concentration (% dry weight) in winged bean as affected by VAM inoculum and P levels for Serdang and Munchong soils

Phosphorus (kg TSP/ha)		0	30	60	
Soil	Mycorrhiza				
Serdang	Alae	1.112	0.973	0.860	
	Scal	1.085	1.063	0.975	
	Gmos	1.162	1.023	1.023	SED
	Gmix	1.168	1.000	0.992	0.0855
	Control	0.105	0.213	0.298	
Munchong	Alae	1.315	1.263	0.750	
	Scal	1.375	1.308	1.063	
	Gmos	1.107	1.232	0.923	LSD 5%
	Gmix	1.692	1.338	1.198	0.17
	Control	0.192	0.197	0.218	

Shoot N concentrations were highest in plants inoculated with mixed species for both Serdang and Munchong soils.

DISCUSSION

The growth of winged bean was greatly enhanced as a result of mycorrhizal associations and addition of P fertilizers in P-deficient soils. As expected, there was, however, variability in effectivity of the different VAM species tested. Abbott and Robson (1981) and Schubert and Hayman (1986) had earlier made similar observations. Several factors have been attributed as being responsible for the differences in the degree of infectivity between species. One factor affecting effectiveness of a particular VAM species is its ability to infect roots of the host plant at a time most appropriate for increased uptake of a deficient nutrient (Abbott and Robson 1984).

In Experiment 1, the decline in shoot production after the fifth harvest in plants treated with *S. calospora* was probably the result of inter-root competition for phosphate, which becomes more serious with time as the roots become clumped at the sides and bottoms of the pot (Sander *et al.* 1977). This effect was successfully overcome by using a larger pot with 5 kg soil (Experiment 2). In this experiment, all five VAM species tested significantly increased dry matter production, especially at 30 kg P ha^{-1}. Dry matter production of winged bean in Serdang soil was increased by 169-197% following treatment with *S. calospora* and *G. mosseae*, respectively. In the higher P-fixing Munchong soil, increase in dry matter was in the range of 131-220% following infection with the mixture of *Glomus* species, and *G. mosseae*, respectively. This clearly shows that mycorrhizal plants in the Serdang soil were larger, but the actual increase as a result of inoculation with VAM was greater in Munchong soil. The effect of mycorrhiza was less in Serdang Soil because, being a better soil, it allows better growth of the control plants, thus reducing comparatively the effects of inoculation with the mycorrhiza.

The effect of *Rhizobium* in both soils was evident at an intermediate level (P_1) of P fertilization. However, the effect of inoculation was less at P_0 and P_2. This is probably due to inefficient nitrogen fixation at P_0 because of insufficient P in the soil. However, the high rates of P applied (P_2) had adverse effects on *Rhizobium*, resulting in the lower yield of these plants at this level (60 kh TSP ha_{-1}) of P fertilization.

Greater effectiveness of *G. mosseae* and *A. laevis* over the other inocula in the Serdang soil could be indicative of (the respective) soil-endophyte and host-endophyte specificity as suggested earlier by Hayman (1982). Superiority of *G. mosseae* over the other inocula could also be attributed to the ability of this fungus to maintain low spore production for a long period of plant growth (Wilson 1984) as well as to its ability to produce rapidly growing and extensive external hyphae (Aldwell and Hall 1986). The less competitive *A. laevis* loses out to *G. mosseae* because of its slower production of external hyphae. Similar observations for these two species have been reported by Aldwell and Hall (1986). *A. laevis* has also been suspected to have a limited life cycle in the host plant (L.K. Abbott and A.D. Robson, *pers. comm.* to I.R. Hall).

In Serdang soil, the beneficial effects of inoculation with *G. mosseae* were further evidenced by the high shoot P and K content of these plants. In Munchong soil, the mixture of *Glomus* species was the most suitable inoculum in stimulating N, P and K shoot concentrations, and hence the growth of winged bean. The higher shoot K concentrations of plants in Munchong than in Serdang soil gave a clear indication of the "dilution effect" (as defined by

Jarrell and Beverly 1981) of plants in the latter soil. Positive growth responses as a result of inoculation with mixed *Glomus* species in Munchong soil indicates probable synergistic effects between these species. The use of mixed inocula (containing more than one endophyte species) has earlier been shown to give more consistent results than those containing a single species (Daft and Hogarth 1983).

VAM and P Recovery

Successful inoculation with these VAM fungi was also shown by the higher recovery of phosphate from highly phosphate-fixing Serdang and Munchong soils, with 437 and 256% P recovery respectively. The growth of winged bean was enhanced by a factor of four as a result of inoculation (Experiment 2). Greater phosphate absorption of VAM arises because of a superior efficiency of P uptake by these fungi from the labile forms of soil phosphate (Mosse *et al.* 1973; Azizah 1991).

Pot Experiments Versus the Field Trial

Although the field trial could only be regarded as a partial success, the results obtained were consistent with results obtained from the pot experiments. This is significant as conditions in the field are very different to those in pots. The volume of available soil is different, and there may be other growth-limiting factors besides insufficient P (Schubert and Hayman 1986). Consistency of results obtained from pot and field trials could also indicate that factors affecting the growth parameters in both these experiments are similar.

Results obtained from the chi-square test indicate two important points: First, there were significant differences in colonization of roots by the four VAM species four weeks after inoculation, indicating variability between VAM species in the rate of root colonization. Second, from six weeks onwards, there was no significant difference in root colonization between these four species, showing the ability of the slow colonizers to compete with the fast colonizers.

The ability to colonize roots rapidly was repeatedly exhibited by *G. mosseae* under both pot and field conditions. In the latter trial, significant growth stimulation of winged bean as a result of association with the mycorrhizal fungi was recorded at 60 kg P ha^{-1}. Significant growth responses from introduced VAM species strongly indicated the ineffectiveness and slow growth of the indigenous VAM fungal species, since the efficiency of these species is a major determinant governing growth responses of plants to VAM treatment in non-sterile soils.

In view of the successful field response to mycorrhizal inoculations it appears feasible in future trials to inoculate legumes in unsterilized soils. The use of mycorrhiza also seems warranted in future trials because: 1. Most Malaysian soils have low inoculum levels of the indigenous VAM species, which cannot compete with superior, introduced mycorrhiza species (Azizah 1986, 1991), 2. In winged bean, optimum performance was demonstrated at the intermediate level of P fertilization, indicating the potential of these mycorrhizal fungi in lowering mineral fertilizer input and hence a form of saving for the farmers. However, more evidence is required of successful field trials in different soil types before the biotechnology of mycorrhizal inoculation can be applied by farmers. Work is now in progress to achieve this goal.

CONCLUSION

These experiments clearly show the importance of time-course studies in elucidating the interactions between mycorrhizal fungi and the host plant. The significant and appreciable growth increases over the entire experiment obtained by inoculating with mycorrhizal fungi, especially *G. mosseae*

and a mixture of *Glomus* species, are sufficiently encouraging to warrant further utilization of these mycorrhizal fungi in other field studies in the humid tropics.

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Effects of Formaldehyde Fumigation and Fytolan Drench on VAM Fungi and Nodulation in Some Leguminous Forest Tree Seedlings in India

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Keywords: formaldehyde fumigation, Fytolan drench, VAM colonization, nodulation, field survival

ABSTRAK

Anak benih 12 spesies pokok kekacang (Acacia caesia, A. farnesiana, A. holosericea, A. leucocephala, A. nilotica, Albizia lebbeck, Dichrostachys cinerea, Leucaena latisiliqua, Prosopis cineraria, Dalbergia latifolia *dan* Pterocarpus marsupium) *dibesarkan dalam formaldehid yang dipewasap/batas Fitolan basah ditapak semaian. Anak benih dalam batas formaldehid yang di pewasap terbantut membesar dan klorotik; mempunyai perkolonian akar VAM yang lemah (18-25.3%) dan densiti spora (3.1 – 0.16 g tanah^{-1}), jumlah bintil yang rendah (3 – 8 tanaman^{-1}) dan biojisim berbintil (100 – 870 mg plant^{-1}); jumlah biojisim (15.5 – 72 g plant^{-1}) dan kadar terus hidup lapangan 31.2 – 40.4%) anak benih adalah sangat rendah. Spesis mikoriza yang diasingkan adalah* Acaulospora bireticulata, G. flasciculatum *dan* G. geosporum. *Sebagai perbandingan, anak benih dari batas Fitolan yang basah menunjukkan pertumbuhan biasa, biojisim yang bertambah (18 – 82.2 f g plant^{-1}) dan kadar terus hidup lapangan lebih tinggi (71 – 86%); penglolonian akar VAM yang sangat menggalakkan (53.4 – 100%) dan jumlah bintil densiti spora lebih tinggi (36 – 82.8 g. tanah^{-1}) dan jumlah bintil yang lebih tinggi (7.4 – 17.6 plant^{-1}) dan biojisim berbintil (195 – 950 mg plant^{-1}) dibandingkan dengan anak benih terkawal. Akar untuk tanaman-tanaman ini mempamerkan perkembangan struktur arbuskular dan vesikular yang sangat menggalakkan. Daripada tujuh spesis VAMF yang direkodkan dari tanah-tanah rizosfera terkawal dan batas basah Fitolan,* A. bireculata, G. fasciculatum *dan* G. geosporum *merupakan spesis dominan. Secara statistik, perbezaan antara rawatan adalah ($p < 0.05$).*

ABSTRACT

*Seedlings of 12 legume tree species (*Acacia caesia, A. catechu, A. farnesiana, A. holosericea, A. leucocephala, A. nilotica, Albizia lebbeck, Dichrostachys cinerea, Leucaena latisiliqua, Prosopis cineraria, Dalbergia latifolia *and* Pterocarpus marsupium*) were raised in formaldehyde-fumigated/Fytolan-drenched beds in a nursery. Seedlings in the formaldehyde fumigated beds had stunted growth and were chlorotic; had poor VAM root colonization (18-25.3%) and spore density (3.1 – 10.6 g. soil^{-1}) and lower nodule number (3 – 8 plant^{-1}) and nodular biomass (100 – 870 mg plant^{-1}); the total biomass (15.5 – 72 g plant^{-1}) and field survival rate (31.2 – 40.4%) of the seedlings were very low. The mycorrhizal species isolated were* Acaulospora bireticulata, Glomus fasciculatum *and* G. geosporum. *In contrast, seedlings form Fytolan-drenched beds showed normal growth, enhanced biomass (18 – 83.2f g plant^{-1}) and higher field survival rate (71 – 86%); intense VAM root colonization (53.4-100%) and higher spore density (36 – 82.8 g soil^{-1}) and higher nodule number (7.4 – 17.6 plant^{-1}) and nodular biomass (195 – 950 mg plant^{-1}) compared with the control seedlings. Roots of these plants exhibited extensively developed arbuscular and vesicular structures. Of the seven VAMF species recorded from the rhizosphere soils of control and Fytolan-drenched beds,* A. bireticulata, G. fasciculatum *and* G. geosporum *were the dominant species. The differences between treatments were statistically significant ($P < 0.05$).*

INTRODUCTION

In India, 68% of the population is rural based and depend on forests for fuelwood, timber, cattle fodder and other minor products. Over-exploitation of forest lands has led to the dwindling of forest cover, causing severe ecological imbalance and environmental disaster. As part of the national effort to open new forests, barren substandard soils and low productivity agricultural lands have been used to raise multi-utility trees through plantation programmes. The seedlings needed for these programmes are usually raised in forest tree nurseries.

Legumes often have double symbiotic associations with *Rhizobium* spp. and mycorrhizal fungi (VAM and/or ECM). Such associations often benefit the leguminous plants through improved P and N supplies and also from N-P interactions (Munns and Mosse 1980). Nitrogen fixation by members of Papilionoideae and Mimosoideae needs high phosphate levels, which could probably be satisfied through mycorrhiza symbiosis. Mycorrhizal fungi not only help the plant itself, but also aid the bacterial system to fix N in the nodular tissue. Hence, legumes with dual symbioses are preferred for forestation programmes in marginal environments.

The use of fumigants and fungicides is an important management practice in nurseries to control root pathogenic fungi, soil-inhabiting insects, nematodes and weed seeds. Properly applied fumigation also usually eliminates the beneficial mycorrhizal population (Kormanik *et al.* 1977; Riffle 1980; Maronek *et al.* 1981; Udaiyan *et al.* 1995) as well as nitrogen-fixing and symbiotic bacteria (Trappe and Strand 1969; Abrahamson 1980; Molina and Trappe 1984; Kishinevsky *et al.* 1992). Since most fungicides are not pathogen-specific, they affect a wide range of non-pathogenic fungi (Bollen 1979; Vyas 1988), including those which are beneficial to plant growth, such as VAMF. VAMF are also affected by fungicide application (Manjunath and Bagyaraj 1984; Kough *et al.* 1987; Fitter and Nichols 1988; Plenchette and Perrin 1992; Sukarno *et al.* 1993; Udaiyan *et al.* 1995).

Studies on the effect of different fungicides, both systemic and non-systemic, on the development of VAM infection do not, however, show consistent overall trends (Trappe *et al.* 1984). Non-systemic fungicides have been reported to be ineffective on VAM (Jalali and Domsch 1975; Sutton and Sheppard 1976; Nemec 1980) to reduce VAM development (Nesheim and Linn, 1969; El-Giahmi *et al.* 1976; Plenchette and Perrin 1992) or, surprisingly, to stimulate it (Sugavanam *et al.* 1994).

The purpose of the present study is therefore to assess the effects of formaldehyde fumigation and Fytolan drench on the VA mycorrhizal and nodular endophytes and their subsequent effects on seedling quality and field survival of some leguminous forest tree species.

MATERIALS AND METHODS

The study was carried out in the nursery in the experimental plot of Bharathiar University, Coimbatore, Tamil Nadu, India. The red alfisol - calcareous soil had a pH of 8.1 and electric conductivity of 0.2 $mScm^{-1}$. Soil nitrogen (N), phosphorus (P) and potassium (K) concentrations were 104, 4 and 380 kg ha^{-1} respectively. Total N and available P were respectively determined by the micro Kjeldahl and molybdenum blue method (Jackson 1973). Exchangeable K was measured using a digital flame photo meter (Jackson 1973). The soil was ploughed to a depth of 50 cm, levelled and 1.5 × 1.5 m nursery beds prepared with 2-m intervals between them.

The treatments of the beds were: a) fumigated with 0.4% formaldehyde applied at the rate of 2 l/m^2 covered with polyethylene sheets for 48 h and then exposed to air for 15 days prior to sowing; b) drenched with 0.2% Fytolan, a non-systemic fungicide with protectant properties containing 88% (w/w) copper oxychloride, applied at the rate of 75ml/m^2 for six hours prior to sowing and; c) untreated control. Each treatment was replicated five times. The plots were arranged in a completely randomised block design.

Fully mature, uniform size, viable seeds of 12 forest tree species (*Acacia caesia* (L.) Willd., *A. catechu* (L.F) Willd., *A. farnesiana* (L.) Willd., *A. holosericea* A. Cunn., *A. leucocephala* (Roxb.) Willd., *A. nilotica* (L.) Willd ex. Del. subsp. *indica* (Benth.) Brenan, *Albizia lebbeck* (L.) Willd., *Dichrostachys cinerea* (L.) Wight & Arn., *Leucaena latisiliqua* (L.) Gills, *Prosopis cineraria* (L.) Douce, *Dalbergia latifolia* Roxb. and Pterocarpus marsupium Roxb.) from the Institute of Forest Genetics and Tree Breeding (IFG &TB), Coimbatore, were sown in nursery beds in June 1992 and watered by irrigation at weekly intervals. Uniform 60-day-old seedlings were transferred to 30 × 12 cm polyethylene bags, each filled with ca. 3 kg soil from the respective seedling beds. Holes were punched in bags for drainage. The bags were arranged closely for sprinkle-irrigation. Samples of feeder root and rhizosphere soil were collected randomly from 10 seedlings for each species and each treatment 60 days after planting.

Root Colonization

Randomly selected root segments were cleaned and stained for assessment of mycorrhizal colonization. The cleared root segments were washed in distilled water, acidified with 5N HCl and stained in trypan blue (0.05% in lactophenol) adopting the technique of Phillips and Hayman (1970). Stained root segments were then examined for the presence of VAM structures, and the percentage of mycorrhizal infection determined by the root slide technique of Read *et al.* (1976).

Spore Population

Total spore count in soil samples was estimated by a modified wet sieving and decanting technique of Gerdemann and Nicolson (1963). Spore population was expressed as the number of individuals per gram of dry soil.

Field Survival Rate

The respective 150-day-old seedlings (100 seedlings for each species from the different treatments) were subsequently transplanted to degraded, barren land at the foot of the Maruthamalai hills, Western Ghats in the Bharathiar University campus in the monsoon month of October 1992. A 4 × 4 spacing was maintained for all seedlings. Data on the field survival of these seedlings were collected in February 1993.

Statistical Analyses

The data were analysed by analysis of variance (ANOVA) and the means were separated by Duncan's new multiple range test ($P < 0.05$). Pearson's coefficient correlations were performed for plant dry weight with nodule number, nodule dry weight, root colonization, spore number and field survival rate.

RESULTS

Soil

The soil at the study area was sandy loam, and low in available nutrients especially phosphorus (4 kg ha^1). However, supplementary fertilizers were not added.

Formaldehyde Fumigation

Seedlings in the fumigated beds were found to be stunted and chlorotic. They had very

poor biomass and stunted growth. Maximum reduction was found in *Prosopis cineraria* followed by *Acacia caesia, A. catechu* and *Pterocarpus marsupium*. After 60 days in polythene bags, VAM root colonization and spore density, the number, size and biomass of nodules and field survival rate of these seedlings decreased significantly ($P < 0.05$) compared with control seedlings. This effect was maximum in *Acacia nilotica* and *Dichrostachys cinerea*. Spores of *Acaulospora bireticulata, Glomus fasciculatum, G. geosporum* and *G. macrocarpum* were isolated from the respective rhizosphere soils.

Fytolan Drench

Seedlings from the Fytolan-drenched soils showed significantly ($P < 0.05$) higher biomass and field survival compared to the control beds. *Acacia caesia, A. holosericea, Leucaena latisiliqua* and *Prosopis cinerea* had increased biomass. Field survival rate increased by 21.7, 14.5 and 12.8% in *Acacia leucocephala, Pterocarpus marsupium* and *Dalbergia latifolia* respectively. VAM root colonization, spore density, legume nodule number and biomass were significantly greater ($P < 0.05$) in *Dichrostachys cinerea, Acacia catechu, A. farnesiana* and *A. holosericea* respectively, than in control seedlings (Table 1). Well-developed VAM structures were observed in treated and control seedlings. Of the seven VAMF species isolated (*Acaulospora bireticulata, A. sporocarpia, Gigaspora margarita, Glomus australe, G. fasciculatum, G. geosporum* and *G. macrocarpum*), *A. bireticulata, G. fasciculatum* and *G. geosporum* were the dominant species, contributing 30, 25 and 20%, respectively, to the total spore count. The field survival rate of the transplanted seedlings from formaldehyde-fumigated, Fytolan-drenched and control soils were 31-40, 71-86 and 64-80%, respectively (Table 1).

A significant positive correlation was established (Table 2) between plant dry weight and nodule dry weight in *Acacia caesia* ($P < 0.001$), *A. catechu* ($P < 0.05$), *A. holosericea* ($P < 0.001$) and *A. nilotica* ($P < 0.05$), but not in the others. The field survival rate of *A. nilotica* and *Pterocarpus marsupium* significantly and positively correlated ($P < 0.05$) with plant dry weight.

DISCUSSION

The results showed that seedlings raised in formaldehyde-fumigated soil were chlorotic and had stunted growth. Their field survival rate was about half that of seedlings from control as well as Fytolan-treated beds. These adverse effects on the quality and performance of seedlings are correlated with a reduction in nodular biomass and poor root colonization by VAMF. Similar results on formaldehyde fumigation in leguminous crops have been reported by Udaiyan *et al.* (1995).

Poor root colonization by VAM was probably due to reduced spore density in the fumigated nursery soil. A similar reduction in VAMF spore density was reported in the rhizosphere of wheat (Hayman 1970) and citrus (Nemec 1980) as a consequence of formaldehyde fumigation. The reduction in nodule number may be due to destruction of the rhizobial population by fumigation (Kishinevsky *et al.* 1992); and the non-availability of sufficient P supply for nodulation (Mosse *et al.* 1976).

It has been suggested that VAMF may play a role in satisfying the high P demand for good nodulation and nitrogen fixation in the control beds (Asimi *et al.* 1980). The synergistic interactions between the bacterium *Rhizobium* and the mycorrhizal endophytes not only enhanced nutrient content in the above-ground plant material, but also seemed to provide well-balanced nutrients to the plants. This subsequently resulted in an improvement in biomass production. Furthermore, mycorrhizal in-

TABLE 1

Effects of formaldehyde fumigation and Fytolan drenching on growth, root colonization, sporulation and field survival of forest tree seedlings

Parameters	Treatments	*Acacia caesia*	*Acacia catechu*	*Acacia farnesiana*	*Acacia holose-ricea*	*Acacia leucoc-ephala*	*Acacia nilotica*	*Albizia lebbeck*	*Dichrostachys cinerea*	*Leucaena latisil-iqua*	*Prosopis cineraria*	*Dalbergia latifolia*	*Pterocarpus marsupium*
Plant d. wt. (g plant)$^{-1}$	Control	58.0 ab	43.0 a	30.0 a	42.5 b	67.0 b	72.8 a	43.2 a	28.0 a	34.1 b	80.0 a	16.2 b	34.0b
	Formaldehyde	55.0 b	40.0 ab	28.0 b	41.0 b	66.5 b	71.0 b	41.3 b	26.3 b	32.4 c	72.0 b	15.5 b	31.0 b
	Fytolan	61.0 a	45.0 a	31.0 a	45.0 a	68.0 a	73.0 a	44.0 a	29.1 a	36.3 a	83.2 a	18.0 a	35.6 a
No. nodules (plant)$^{-1}$	Control	14.6 a	18.0 a	12.0 b	16.1 b	20.3 a	21.4 a	8.2 a	20.3 a	19.4 a	15.4 a	7.5 a	9.4 a
	Formaldehyde	4.8 b	6.0 b	8.0 c	4.4 b	7.5 c	6.0 b	3.0 c	6.0 b	7.1 c	4.1 b	5.2 b	4.2 c
	Fytolan	12.0 a	17.6 a	14.3 a	15.0 a	13.0 a	16.4 a	7.4 b	12.2 a	11.0 b	16.3 a	8.1 a	11.3 a
Nodule d. wt (mg plant)$^{-1}$	Control	400 ab	380 b	475 b	900 b	193 a	250 a	200 a	620 a	700 a	195 a	285 b	270 a
	Formaldehyde	370 b	320 c	460 c	870 c	180 b	100 b	120 b	400 b	520 b	170 b	150 c	250 b
	Fytolan	430 a	427 a	510 a	950 a	195 a	280 a	210 a	630 a	725 a	200 a	300 a	300 a
Root colo-nization (%)	Control	58.3 a	98.4 a	70.0 a	40.7 b	90.6 a	100.0 a	87.4 b	37.4 b	57.5 a	85.2 a	77.3 a	81.2 a
	Formaldehyde	21.0 b	18.0 c	21.5 b	22.3 c	25.3 b	23.1 b	19.0 c	18.3 c	16.6 b	25.0 b	22.0 b	16.0 b
	Fytolan	53.4 a	89.6 b	77.2 a	56.2 a	95.1 a	100.0 a	94.3 a	56.0 a	56.0 a	91.3 a	85.2 a	83.3 a
No. of spores (g soil)$^{-1}$	Control	70.2 a	41.1 a	50.6 a	82.8 a	48.0 a	27.5 a	42.2 a	94.2 a	55.3 a	40.7 a	38.6 b	37.7 b
	Formaldehyde	5.1 b	4.5 b	8.3 b	3.1 c	6.2 b	8.3 c	8.4 b	10.6 c	5.7 b	9.1 b	7.3 c	6.3 c
	Fytolan	62.6 a	58.2 a	60.6 a	70.4 b	58.5 a	36.0 a	48.1 a	82.8 b	50.6 a	44.5 a	43.4 a	49.4 a
Field Survival rate (%)	Control	68.3 a	76.8 b	80.3 a	69.0 a	64.3 b	80.6 a	69.0 a	73.0 a	80.0 a	78.3 a	65.2 b	68.6 b
	Formaldehyde	38.2 b	32.3 c	40.4 b	34.5 b	37.2 c	31.2 b	38.6 c	39.2 b	38.3 b	37.6 b	32.1 c	37.2 c
	Fytolan	71.0 a	82.0 a	84.2 a	72.4 a	86.0 a	73.5 a	82.8 a	78.5 a	82.2 a	79.2 a	78.0 a	83.1 a

Means within a parameter followed by the same superscript are not significantly different according to Duncan's new multiple range test ($P < 0.05$)

TABLE 2
Pearson's correlation coefficient (r) for plant dry weight (PDW) with nodule number (NN), nodule dry weight (NDW), root colonization (RC), spore number (SN) and field survival rate (FSR)

Sl. Tree Species	PDW × NN	PDW × NDW	PDW × RC	PEW × SN	PDW × FSR
1. *Acacia caesia*	+0.6053	+1.0000**	+0.7992	+0.8076	+0.9007
2. *A.catechu*	+0.9056	+0.9989*	+0.8734	+0.9957	+0.7513
3. *A. farnesiana**	+0.9993f	+0.9142	+0.9771	+0.9869	+0.9682
4. *A. holosericae*	+0.7302	+1.0000**	+0.9814	+0.6880	+0.8332
5. *A. leucocephala*	+0.2497	+0.8305	+0.7925	+0.8664	+0.9679
6. *A. nilotica*	+0.9152	+0.9978*	+0.9958	+0.9773	+0.9985*
7. *Albizia lebbeck*	+0.9064	+0.9818	+0.9718	+0.9880	+0.9849
8. *Dichrostachys cinerea*	+0.5397	+0.9351	+0.9933	+0.8645	+0.9635
9. *Leucaena latisiliqua*	+0.2392	+0.8844	+0.8881	+0.7754	+0.8422
10. *Prosopis cineraria*	+0.9770	+0.9922	+0.9805	+0.9832	+0.9658
11. *Dalbergia latifolia*	+0.8391	+0.7766	+0.7915	+0.7963	+0.8781
12. *Pterocarpus marsupium*	+0.9961	+0.9585	+0.9485	+0.9964	+0.9993*

*,**Correlations are significant at P = 0.05 and 0.001 respectively.

fection has also been reported to increase shoot nitrogen content in nodulated plants (Ross and Harper 1970; Ross 1971). This probably explains the normal growth of the control seedlings.

In general, fungicides are less damaging/deleterious to mycorrhiza population than are fumigants. Nesheim and Linn (1969) suggested that stunting of seedlings can be avoided by using fungicides that eliminate root pathogens but are harmful to mycorrhizal fungi. Sugavanam *et al.* (1994) have reported that Fytolan promoted VAM root colonization, rhizosphere spore population and nodulation in *Arachis hypogaea*. In the present study, drenching of the nursery beds with Fytolan was found to increase endomycorrrhizal colonization and *Rhizobium* nodulation in the legume seedlings. But the extent of increase varied among the host species. The differential response to treatments probably reflects the difference in the genetic constitution as well as the microfloral composition in the rhizosphere of the host species. The higher root colonization and spore density in the Fytolan-treated beds is probably due to the following reasons: i) increased survival of VAM fungal propagules at seedling emergence stage, made possible by the suppression of microbes antagonistic to VAMF (Groth and Martinson 1983; Afek *et al.* 1990; Hetrick and Wilson 1991); ii) The insensitivity of certain *Rhizobium* strains to fungicides (Chiranjeevi 1982; Kataria *et al.* 1985; Radhakrishnan and Chatrath 1989; Singh and Agarwal 1990); iii) The synergistic interaction between *Rhizobium* and the mycorrhizal endophytes, where the mycorrhiza fungi enhance P availability for greater nodule formation (Kucey and Paul 1982).

Results from the present study also showed that seedlings with high biomass with roots extensively colonized by the VA mycorrhiza fungi also have higher field

survival rate. Seedlings from Fytolan-drenched beds in particular showed significantly higher field performance than the control. The effective synergistic interactions between the symbionts could probably have provided the necessary prerequisites for the high performance in the field environment. Presence of VAM probably helps to alleviate drought stress during transplanting (Michelsen and Rosendahl 1990) and enhances seedling growth, vigour and survival after transplanting (Brandeau 1970; Biermann and Lindermann 1983). Fytolan drench favours the establishment of the VAM and nodular endophytes of legumes in nutrient-deficient soils and should therefore be employed in nursery management along with other cultural practices for the production of high quality seedlings. The very hazardous chemical, formaldehyde, is not recommended for use in the nursery management practices, if it can be avoided.

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Charge Characteristics in Relation to Mineralogy of Selected Soils from South-east Asia

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Keywords: charge characteristics, mineralogy, weathering, X-ray diffraction

ABSTRAK

Satu kajian mengenai mineralogi dan ciri-ciri cas telah dijalankan terhadap 7 jenis tanah dari Filipina, Indonesia dan Malaysia. Tanah-tanah tersebut termasuk 1 Entisol, 3 Alfisol, 1 Andisol dan 2 Oksisol. Smektit, mika dan kuarza didapati wujud di bahagian lempung pada tanah Entisol. Smektit dan kaolinit ialah mineral-mineral dominan di tanah Alfisol. Kebanyakan mineral di tanah Andisol ialah haloisit, manakala kaolinit dan oksida banyak didapati di tanah Oksisol. Kuantiti kaolinit meningkat dari bawah ke permukaan tanah, sedangkan kuantiti haloisit semakin menurun. Ini menunjukkan haloisit telah ditukarkan kepada kaolinit semasa proses luluhawa. Perbezaan mineralogi bagi tanah yang berlainan adalah jelas dipengaruhi oleh perbezaan ciri-ciri cas. Tanah yang mengandungi smektit (Entisol dan Alfisol) mempunyai cas negatif yang tinggi. Tanah Andisol mengandungi haloisit dan tanah Oksisol mengandungi kaolinit dan oksida, mempunyai jumlah cas positif yang tinggi tetapi jumlah cas negatif yang sederhana. Ketersediaan Ca dalam tanah bergantung kepada Ca tukarganti dan keupayaan pertukaran kation berkesan.

ABSTRACT

The mineralogy and charge characteristics of 7 soils from the Philippines, Indonesia and Malaysia were studied. The soils consisted of an Entisol, 3 Alfisols, an Andisol and 2 Oxisols. Smectite, mica and quartz were present in the clay fraction of the Entisol. In the Alfisols, smectite and kaolinite were the dominant minerals. The Andisol was dominated by halloysite, whereas the Oxisols were dominated by kaolinite and oxides. The amount of kaolinite increased towards the surface, while halloysite decreased, indicating the transformation of halloysite to kaolinite during the course of weathering. Differences in mineralogy of the various soil types were reflected clearly in the differences in charge characteristics. Soils with smectite (Entisol and Alfisol) had high a negative charge. The Andisol, which contained halloysite, and the Oxisol, with kaolinite and oxides, had high amounts of positive charge, but moderate amounts of negative charge. The availability of Ca in the soils depended upon exchangeable Ca and the effective cation exchange capacity (ECEC).

INTRODUCTION

Many soils in the Philippines and Indonesia and some soils in Malaysia are derived from volcanic rocks of recent to Pleistocene age. Depending on the age and composition of the parent rock, and the stage of weathering, volcanic soils in the tropics contain allophane, halloysite, smectite, kaolinite, goethite and gibbsite (Eswaran 1979; Delvaux *et al.* 1989). Volcanic soils classified as Andisols are known to contain large amounts of halloysite (Mohr *et al.* 1972;

Allen and Hajek 1989). Imogolite and X-ray amorphous hydrous aluminosilicates (collectively known as allophane) are common in these soils (Wada 1989). Geographic distribution of allophane and imogolite has been connected with areas of recent volcanic activity throughout the Pacific ring.

The charge of Andisols containing allophane and imogolite is pH dependent (Okamura and Wada 1983). The cation exchange capacity (CEC) is known to increase with increasing soil pH and/or ionic strength (Gillman and Hallman 1988). For instance, the CEC increased when fertilizer such as sulphate of ammonia was applied, via replacement of OH^- by SO_4^{2-} (Guadalix and Pardo 1991). The CEC of these soils can be determined accurately by Ca or Ca plus Al adsorption (Gillman and Sumpter 1986) or by a compulsive exchange method (Gillman and Hallman 1988). Such an estimate gives a true CEC value under field conditions; this information is considered useful in soil management. The charge and ion retention properties chiefly govern the soil cation dynamics. In view of the lack of baseline data for these soil types, the objectives of this research were to characterize the mineralogy of a range of volcanic soils, occurring under different climatic conditions in the Philippines, Indonesia and Malaysia, and to establish the relationship between their mineralogy and charge characteristics.

MATERIALS AND METHODS

The Soils

An Entisol (Philippines), 3 Alfisols (Philippines), an Andisol (Indonesia) and 2 Oxisols (one each from the Philippines and Malaysia) were examined in the field, classified (Soil Survey Staff 1990) and sampled. Table 1 gives their location, annual rainfall, rock type and geologic

TABLE 1
Location, annual rainfall, rock type, geologic age and classification of soils from Indonesia, Malaysia and the Philippines

Country	Location	Annual Rainfall (mm)	Rock Type	Geologic Age	Classification
Philippines	Setio Bueno, Tarlac	1986	basalt	Holocene	Lithic Ustorthents
	UPLB, Laguna	2006	dolerite, tuff	Pleistocene	Typic Hapludalfs
	Rosario, La Union	2297	basalt	Pleistocene	Typic Hapludalfs
	VISCA, Leyte	2499	basalt	Pleistocene	Typic Palendalf
	CMU, Bukidnon	1828	basalt	Pleistocene	Kandiustalfic Eutrustox
Indonesia	Carita 11, Java	2874	basalt	Tertiary	Typic Hapludands
Malaysia	Kuantan, Pahang	2757	basalt	Tertiary	Typic Acrudox

TABLE 2
Chemical properties of soils from Indonesia, Malaysia and the Philippines

Soil	Hor	pH(1:1) (H_2O)	pH_o	Exch Ca	ECEC	B.S	Fe_2O_3	C_{org}	Clay
				$cmol_c/kg$			(%)		
Entisol	A	5.2	2.7	16.3	23.5	97.2	2.4	1.8	28.6
Alfisol	Ap	6.2	3.7	25.1	34.4	99.9	2.1	1.1	37.7
	Bt_2	5.3	nd	29.7	42.7	99.3	1.6	1.3	53.2
Andisol	A_1	4.3	3.5	1.4	2.3	95.6	7.3	1.4	76.4
	Bw_2	4.7	nd	1.3	3.6	44.3	7.8	1.0	73.5
Oxisol	A	5.7	3.3	12.4	17.1	99.4	8.6	3.3	65.1
(Eutrustox)	Bt_2	4.7	nd	6.7	8.1	91.4	10.6	1.3	74.3

nd = not determined

age. Four soils were selected for detailed investigation into their mineralogy and charge properties. Chemical properties are given in Table 2. Note that for Table 2 both the Alfisol (UPLB) and Oxisol (CMU) were from the Philippines, and the Entisol is a shallow soil without B and C horizons. In addition, the Oxisol is a special type, Eutrustox, which has a very high base value (91.4 – 99.4%) compared with normal Oxisols, which have a base value of less than 35%.

Soil Analysis

The pH of a 1:1 solution of soil in water was determined after 1 h of intermittent shaking and standing overnight. Exchangeable Ca and Mg were extracted by 1 M NH_4OAc and determined by atomic absorption spectrophotometry, while exchangeable K and Na in the same extract were determined by flame photometry. Exchangeable Al was extracted by 1 M KCl and determined colorimetrically (Barnhisel and Bertsch 1982). Effective cation exchange capacity (ECEC) was calculated as the sum of basic exchangeable cations and exchangeable Al, while base saturation (BS) was calculated on the basis of ECEC. Free iron oxide content was determined by the method of Mehra and Jackson (1960). The Walkley-Black method (Nelson and Sommers 1982) was used to measure organic carbon (C_{org}).

Clay content of the soils was determined by the pipette method of Day (1965). To obtain the clay, the soil was first treated with H_2O_2 to remove organic matter. It was later dispersed with dilute Na_2CO_3 solution. This clay (< 2μm) was later used to study the mineralogy of the soils by X-ray diffraction (XRD) and transmission electron microscopy (TEM). The XRD analysis was conducted by an automated Phillips diffractometer equipped with a graphite monochromator, operated from 3 to 50 degrees 2-theta, using Cu Kα radiation and scanning speed of half a degree per minute. The X-ray diffraction analysis was carried out on Mg-saturated and glycolated samples.

Charge characteristics of the soils were determined by the method of Gillman and Sumpter (1986). In this method, negative charge as measured by Ca adsorption was termed CEC_B, while that measured by Ca and Al adsorption was termed CEC_T. The positive charge as measured by Cl adsorption was termed AEC. Soil weathering index (WI) was calculated (at soil pH) as follows (Tessens and Shamshuddin 1983):

$$WI = \frac{\text{negative charge} - \text{positive charge}}{\text{negative charge}} \times 100$$

The negative charge (CEC_B) and the positive charge required for WI determina-

tion were estimated from the charge curves. PH_o, defined as the pH of the variable charge colloid at which the net charge is zero, was determined by the method of Gillman and Sumpter (1986).

Extraction of Soil Solution and Analysis

Distilled water was added to the air-dried soils and the wetted soils were subsequently incubated for 1 day at a matric suction of 10 kPa (Menzies and Bell 1988). This study assumed that a state of equilibrium was reached between the liquid and solid phase of the soils during the incubation period. Soil solutions were extracted by centrifugation at 2000 rpm for 1 h using specially designed centrifuge tubes. The pH and electrical conductivity (EC) of each soil extract were immediately determined from 2-ml subsamples. The remainder of the extract was stored at 5°C for later determination of Ca, Mg, K, Na, Fe, Al, S and P by inductively coupled plasma atomic emission spectroscopy (ICPAES).

RESULTS AND DISCUSSION

Soil Types

Differences in the lithology, age of parent material, climate, drainage conditions and vegetation for the soils in the different regions (Table 1) were reflected by the differences in their chemical properties (Table 2), as the soils had undergone different rates of chemical weathering. Hence, several groups of soils ranging from Entisol to Oxisol are found in the region. According to their chemical properties and profile morphology, the least weathered of all the study soils was sited at Setio Bueno, Tarlac, the Philippines (Entisol), while the most weathered soil was found at Bukidnon, the Philippines and Kuantan, Malaysia (Oxisol). The other soils, which had high basic exchangeable cations, were classified as Alfisols (Philippines). The soil which had low bulk density and a high amount of amorphous materials was classified as an Andisol (Indonesia). The Oxisol and Andisol also contain high amounts of Fe_2O_3.

Mineralogy

Overall, the peaks on the X-ray diffractograms of the soils were clear although the clay samples were likely to be coated with the amorphous materials (including allophane and/or sesquioxides). Furthermore, the low scanning speed was slow enough to allow differentiation of the individual minerals on the X-ray diffractograms. Although allophane is common in volcanic soils (Mohr *et al.* 1972; Wada 1989) it was not confirmed by the XRD analysis or TEM observation.

The X-ray diffractogram of the Entisol had reflections at 1.52, 1.0, 0.713, 0.5, 0.445, 0.426, 0.418, 0.356, 0.334, 0.245 and 0.212 nm (*Fig. 1*). The strong and clear reflections at 0.426 and 0.334 nm indicated the presence of quartz in the clay fraction. Mica (1.0, 0.5 nm), smectite (1.52 nm) and kaolinite (0.713, 0.356 nm) were also present, but in lesser amounts. A small amount of goethite (0.418, 0.245 nm) and hydrated halloysite ($Al_2Si_2O_5(OH)_4.2H_2O$) were present. The presence of the hydrated halloysite was shown by the 1.0, 0.445 and 0.346 nm reflections (Dixon 1989). The TEM micrograph (*Plate 1A*) gives a visual illustration of the presence of kaolinite, mica and/or smectite in the soil. The smectite in the soil was probably an alteration product of mica weathering under impeded drainage conditions (Velde 1992). This smectite can be interstratified with the hydrated halloysite as shown by Delvaux *et al.* (1989) in volcanic soils of Cameroon.

Smectite, halloysite, kaolinite and goethite were present in the topsoil of the Alfisol (*Fig. 1*). The 1.5 – 1.8 nm peak was strong and intense, suggesting that smectite

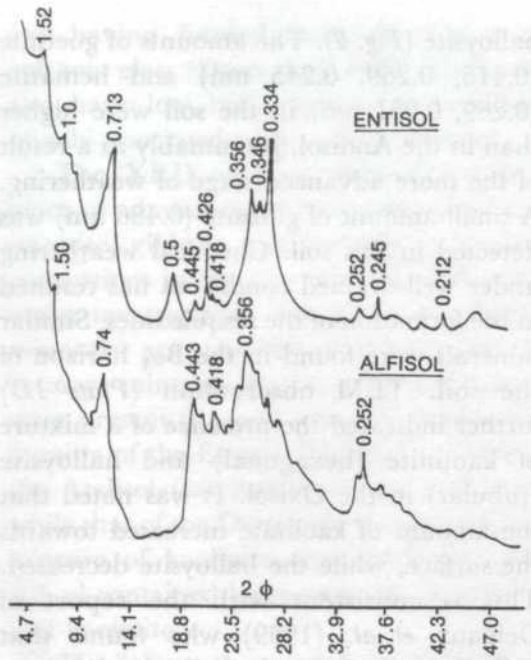

Fig. 1 X-ray diffraction patterns of Mg-saturated clay fraction from the A horizon of the Entisol and Alfisol.

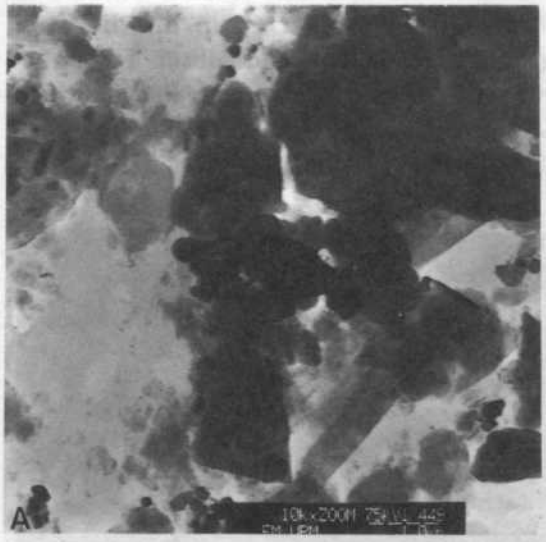

Plate 1A. TEM micrograph of the clay fraction from the A horizon of the Entisol

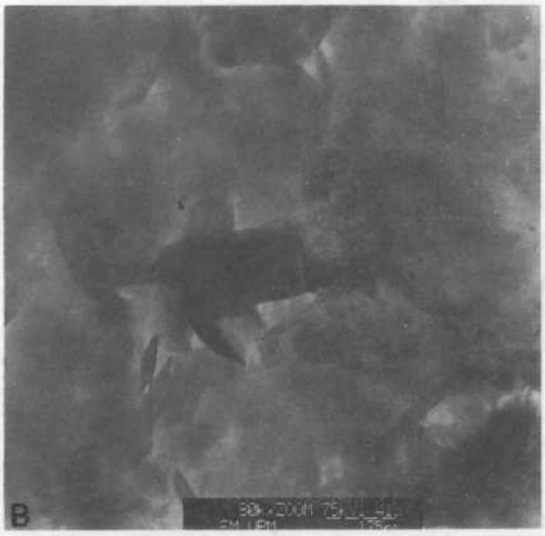

Plate 1B. TEM micrograph of the clay fraction from the Ap horizon of the Alfisol

was present in large amounts. This is consistent with the high ECEC value (*Table 2*) and is a common feature of a volcanic soil with moderately well drained conditions existing under a udic moisture regime. The presence of amorphous materials in the clay sample tended to reduce the sharpness of the XRD peaks. *Plate 1B* shows the occurrence of halloysite, kaolinite and/or smectite being coated by the amorphous materials. The halloysite in the soil was similar in morphology to the ferruginous halloysite from Hokkaido, Japan (Wada and Mizota 1982).

The X-ray diffractogram of the Andisol (*Fig. 2*) showed the dominance of halloysite (0.73, 0.445 nm) with minor amounts of kaolinite (0.358 nm), goethite (0.418, 0.269, 0.245 nm) and hematite (0.270, 0.252 nm). The sample from the C horizon (not shown) gave a similar XRD pattern, indicating a similar mineralogy in both horizons. The peaks 0.73 and 0.445 nm belong to dehydrated halloysite, which has a formula of $Al_2Si_2O_5(OH)_4$ (Dixon 1989). This halloysite is common in youthful soil developed from volcanic rock (Dixon 1989; Allen and Hajek 1989). TEM observation further indicated that the clay fraction was dominated by halloysite, without clear manifestation of the presence of allophane and/or imogolite (*Plate 1C*). Further TEM observations (not illustrated here) suggest

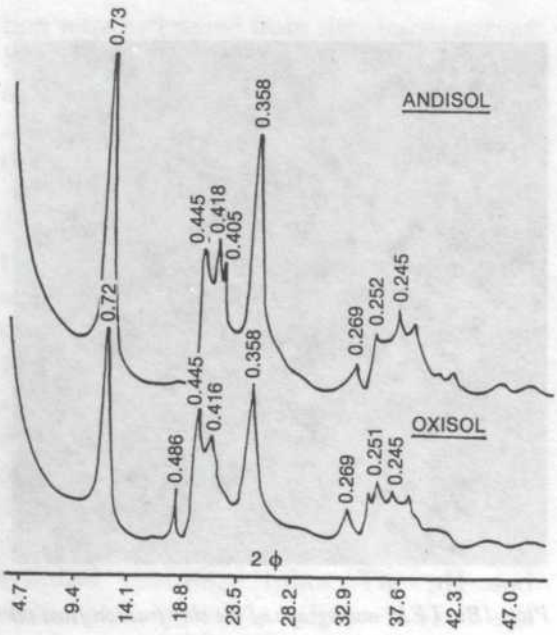

Fig. 2 X-ray diffraction patterns of Mg-saturated clay fraction from the A horizon of the Andisol and Oxisol

that halloysite was more dominant in the subsoil than in the topsoil.

The XRD peaks on the diffractogram of the Oxisol were different from those of the Andisol in that clear peaks were observed at 0.72 and 0.358 nm, showing that kaolinite was more dominant than halloysite (*Fig. 2*). The amounts of goethite (0.416, 0.269, 0.245 nm) and hematite (0.269, 0.251 nm) in the soil were higher than in the Andisol, presumably as a result of the more advanced stage of weathering. A small amount of gibbsite (0.486 nm) was detected in this soil. Chemical weathering under well-drained conditions has resulted in the formation of the sesquioxides. Similar minerals were found in the Bo_4 horizon of the soil. TEM observation (*Plate 1D*) further indicated the presence of a mixture of kaolinite (hexagonal) and halloysite (tubular) in the Oxisol. It was noted that the amount of kaolinite increased towards the surface, while the halloysite decreased. This is consistent with the report of Delvaux *et al.* (1989) who found that halloysite in volcanic soils in Western Cameroon was formed earlier than kaolinite in the weathering sequence.

Clear X-ray reflections at 0.404 and 0.248 nm were observed in the diffractograms of the Andisol and Alfisol. The mineral which gives the reflections was identified as cristobalite, which is commonly associated with volcanic deposits of Tertiary

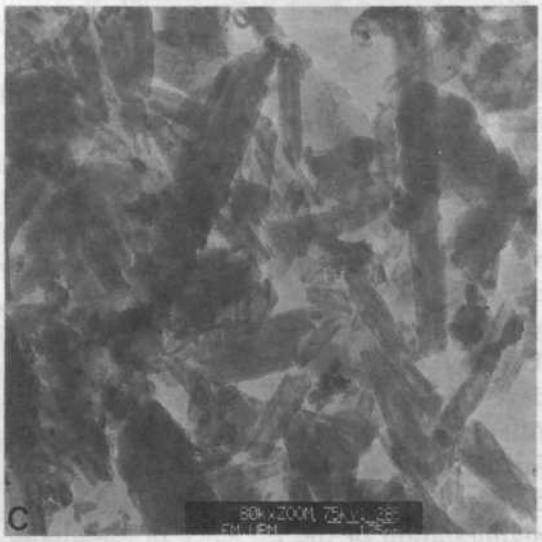

Plate 1C. TEM micrograph of the clay fraction from the A horizon of the Andisol

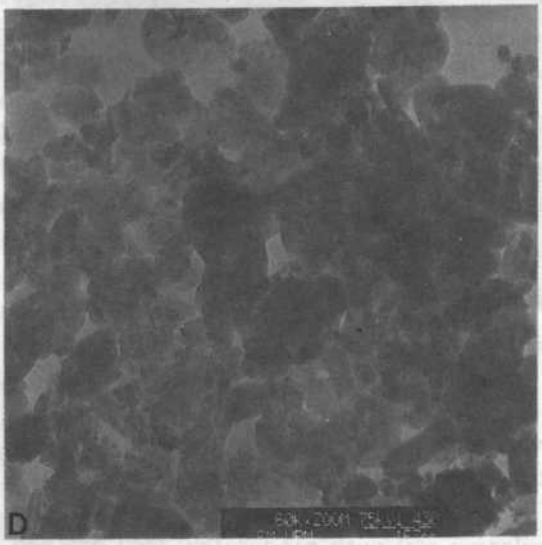

Plate 1D. TEM micrograph of the clay fraction from the A horizon of the Oxizol.

age having formed from dissolution of volcanic glass (Drees *et al.* 1989). Both soils also have low bulk density, an attribute usually associated with andic properties.

The XRD analyses showed the presence of admixtures of halloysite, kaolinite, smectite, gibbsite and goethite in various proportions in the clay fraction of the soils under investigation. The majority of those minerals are variable-charge minerals. Primary minerals such as mica and quartz were present in small amounts in the clay fraction of the Entisol. The clay fraction of the Andisol was dominated by halloysite, while that of the Oxisol was dominated by a mixture of kaolinite and halloysite. The Alfisol contained large amounts of smectite and kaolinite.

Fig. 3 shows the suite of clay minerals in the different soil types arranged in order of decreasing abundance. Volcanic materials first weather to form either Andisol or Entisol, depending on the mineralogical composition and moisture regime. According to Mohr *et al.* (1972), volcanic glass under well-drained conditions first changes to allophane, which on further weathering is transformed to halloysite, and subsequently to kaolinite. This transformation can be visualized in the development of an Andisol.

In the other pathway, mica in the Entisol first weathers to smectite. Further weathering results in a complete destruction of mica and the formation of smectite, which in turn weathers to halloysite and/or kaolinite. Such a sequence typifies the development of an Alfisol. In the Oxisol, kaolinite, oxides and halloysite are present, but smectite is absent. We found that the amount of goethite and hematite increased with weathering. Additionally, kaolinite in the Alfisol, Andisol and Oxisol increased towards the surface, while halloysite decreased. These observations support the scheme of mineral weathering shown in *Fig. 3.*

Charge Properties

The CEC_B of the Entisol and Alfisol was high, with values comparable to those reported by Gillman and Hallman (1988) for the volcanic soils of Papua New Guinea. The high negative charge in the Entisol and

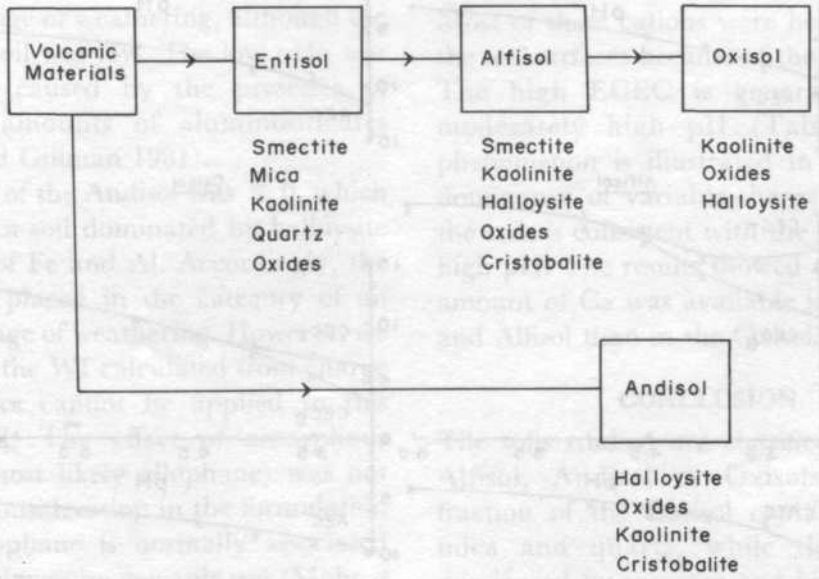

Fig. 3. Soil minerals arranged in order of decreasing abundance in the various soil types

Alfisol was attributed partly to the presence of smectite in the soils (*Fig. 4*). The ECEC of these two soils was high (Table 2). The CECB value of both soils increased as the pH increased. CECT appeared to increase in the Entisol and Alfisol at pH below 3.5 (*Fig. 4*). A portion of the Al measured in the soil solution could have been dissolved from the inner part of the phyllosilicates (smectite or mica) rather than on the exchange sites. At very low pH some of the soil minerals are no longer stable, and Al in the minerals readily goes into the soil solution.

The Andisol had a net positive charge at the soil pH of 4 (*Fig. 4*). Halloysite was the dominant clay mineral in the soil (*Fig. 2*; *Plate 1C*). The changes of CEC_B and AEC with pH were similar to those reported for the volcanic soil with some allophane and/or imogolite (Okamura and Wada 1983). Like the Entisol and Alfisol, the CEC_T of the Andisol increased below pH 3.5. The high positive charge present in the soil was partly caused by the Fe oxides (goethite and hematite), as indicated by their 7.3% content in the topsoil (Table 2). On the other hand, the Entisol and Alfisol, which had quartz, mica and smectite with minor amounts of kaolinite, exhibited different charge characteristics to those of the Andisol. The negative charge in the Entisol and Alfisol was, however, higher than the positive charge at the soil pH.

The positive charge in the Oxisol was slightly lower than in the Andisol. Additionally, the negative charge in the soil was low and is attributed partly to the dominance of low activity clays. XRD analyses *(Fig. 2) and TEM observations*

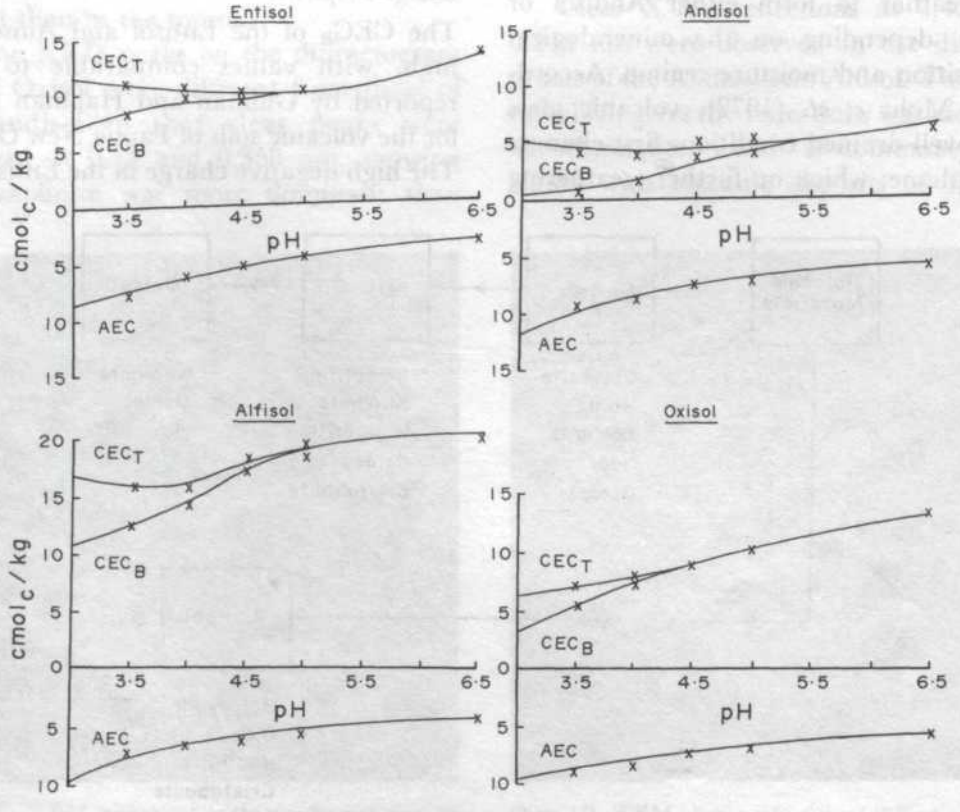

Fig. 4. Charge characteristics of the Entisol, Alfisol, Andisol and Oxisol

(Plate 1C and 1D) showed that kaolinite, halloysite, hematite and goethite were present in large amounts. The high amount of Fe_2O_3 contributed a certain amount of positive charge to the soil surfaces. The CEC_B and AEC of the topsoil are quite similar to those of the Andisol. However, the CEC_T did not increase below pH 3.5. The positive charge in the Bo_4 horizon of the Oxisol (data not shown) was higher than the negative charge at pH of 3.0 to 6.5. This charge pattern was similar to that of the soil at Kuantan, Malaysia. The Kuantan soil, classified as Typic Acrudox (Tessens and Shamshuddin 1983), is one of the most highly weathered soils in Malaysia.

The weathering index (WI) values for the Entisol and Alfisol were high, with values ranging from 100 to 50. The high value means that the soils are in the recent or intermediate stage of weathering (Tessens and Shamshuddin 1983). The low pH_o is directly related to the high WI and is characteristic of newly developed soils (Gallez *et al.* 1976; Uehara and Gillman 1981).

The WI value of the Oxisol was 50 – 0, which fits very well into the category of an advanced stage of weathering, although the pH_o of the soil was low. The low pHo was presumably caused by the presence of significant amounts of aluminosilicates (Uehara and Gillman 1981).

The WI of the Andisol was < 0, which is typical of a soil dominated by halloysite and oxides of Fe and Al. Accordingly, the soil can be placed in the category of an advanced stage of weathering. However, we believe that the WI calculated from charge characteristics cannot be applied to this kind of soil. The effect of amorphous materials (most likely allophane) was not taken into consideration in the formulation of WI. Allophane is normally associated with relatively young volcanic soil (Mohr *et al.* 1972).

Soil Solution Attributes

The soil solution Ca concentrations in the soils of the Philippines were high, but low in soils from Indonesia and Malaysia. The highest value of soil solution Ca concentration was observed in the Bt_2 of the Alfisol. The high value of the soil solution was consistent with the high exchangeable Ca in that horizon; the exchangeable Ca in the Bt_2 horizon of the Alfisol was 29.7 $cmol_c/kg$ (Table 2). The relationship between the soil solution Ca concentration and exchangeable Ca in the soils is given by this equation:

$$Ca_{sol} = 0.79 + 41.40\ Ca_{exch},\quad (r = 0.75,\ p < 0.05)$$

Soil solution Ca concentration was related to the ECEC (calculated on the basis of 1 kg clay) by this equation:

$$Ca_{sol} = -2.70 + 11.00\ ECEC,\quad (r = 0.78,\ p < 0.01)$$

Although the exchangeable Ca in the soils was high, not all the Ca was released into the soil solution (for Philippine soils). Most of these cations were held tightly by the soil surfaces because of the high ECEC. The high ECEC is generated by the moderately high pH (Table 1). This phenomenon is illustrated in *Fig. 4*. The dominance of variable-charge minerals in the soils is consistent with the high CEC at high pH. The results showed that a higher amount of Ca was available in the Entisol and Alfisol than in the Oxisol.

CONCLUSION

The soils studied are classified as Entisol, Alfisol, Andisol or Oxisols. The clay fraction of the Entisol contains smectite, mica and quartz, while the Alfisol is dominated by smectite and kaolinite, with minor amounts of halloysite and goethite.

Halloysite is abundant in the Andisol, while the Oxisol contains a mixture of kaolinite and halloysite with significant amounts of sesquioxides.

The mineralogy of the soils significantly affects their charge characteristics. The Entisol and Alfisol have a high negative charge, but the Andisol has a high positive charge. Generally, the charge characteristics of the Andisol are similar to those of the Oxisol. The soil solution Ca is correlated to the exchangeable Ca and the charge characteristics. The availability of Ca is therefore governed by the amount of exchangeable Ca and the ECEC of the soils.

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Effect of Water Depth, Seedling Age, and Day Length on Elongation Induced by Short-duration Flooding Treatment in Rice

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ABSTRAK

Percubaan yang dijalankan terhadap usia anak benih dan kedalaman air yang berbeza menunjukkan bahawa anak benih jangkamasa pendek, iaitu berusia tiga minggu yang direndam selama tujuh hari, berkesan dalam menilai potensi pemanjangan dalam varieti padi air dalam. Kedalaman air 90-100 cm adalah mencukupi untuk membuktikan keupayaan pemanjangan dan anak benih berusia tiga minggu untuk memberi perbandingan yang lebih baik antara varieti moden memanjangan dan varieti moden tak memanjang. Varieti menghasilkan kepanjangan ruas yang maksimum di bawah keadaan hari yang pendek sementara ruas yang paling pendek diperolehi dalam rawatan hari yang panjang. Pemanjangan ruas mungkin hanya disebabkan oleh kesan kepanjangan hari atau kesan lindungan terhadap asimilasi. Percubaan selanjutnya perlu diadakan untuk mengesahkan penemuan ini.

ABSTRACT

Experiments conducted on seedling age and different water depths revealed that short-duration, 7-day flooding of 3-week-old seedling was effective in assessing elongation potential in deepwater rice varieties. Water depths of 90-100 cm were sufficient to express elongating ability and 3-week-old seedlings gave better contrast between elongating modern varieties and non-elongating modern varieties. Maximum internode length was under short-day-length conditions while shortest internodes were obtained in the long day treatment. Internode elongation may be due only to the signal effect of day length or the shading effect on assimilation. Further experiments should be conducted to confirm these findings.

INTRODUCTION

Survival of plants during flooding depends on various factors such as age of seedlings, water depth and day length (Gomosta 1985). Attempts have been made to screen varieties for elongation ability under conditions of prolonged flooding, but the disadvantage of this method is the lack of control on survival of the non-elongating plants. This leads to inaccuracy in observation and progeny testing of all individuals. In order to overcome problems of testing under prolonged flooding, short-duration flooding treatment is preferred. Khan *et al.* (1987) observed maximum elongation within 24 hours of submergence and suggested that shorter-period flooding may prove fruitful. Thakur and HilleRisLambers (1988) and Dwivedi (1992) found short-duration flooding (7 days' submergence) useful to compare hybrid populations before and after flooding treatments. The available information on the effect of seedling age, water depth and day length is adequate. Therefore, the present study was undertaken to determine the appropriate

water depth and seedling age for testing elongation ability under short-duration flooding suitable for genetic studies. In addition, the effect of day length treatment on elongation was studied.

MATERIALS AND METHODS

Optimum Water Depth for Testing Rapid Elongation in Deepwater Rice Varieties

Experiment 1: Twelve varieties, including IR42 and BKNFR76106-16-0-1 (non-elongating semi-dwarf); NDGR207, Bhatin and IR28273-R-R-R-29-38-2-3-3 (non-elongating tall); IR11141-6-1-4 and IR11288-B-B-69-1 (elongating modern varieties); IR40905-11-3-1-2-3-2 and Leb Mue Nhang 111 (elongating tall); Baisbish, Barogar and Kalaungi (floating rice), were used to determine the optimum water depth for testing rapid elongation at the seedling stage (Table 1). The experiment was conducted at the International Rice Research Institute (IRRI). Plants were raised in small plastic pots of size 5 × 5 × 5 cm. The experiment was laid out in a split plot design with three replications with five water depths (main plots) and twelve entries (sub-plots).

Three-week-old seedlings were transferred to a concrete tank where different water depths (80, 90, 100, and 120 cm) were introduced and maintained. Plants were submerged for 7 days as suggested by Thakur and HilleRisLambers (1988). Plant height from the base of the culm to the tip of tallest leaf was recorded before and after

TABLE 1
Varietal mean for percent elongation at various water depths

Variety	Percent elongation at various water depths* (cm)					
	80	90	100	110	120	Control (no flooding)
Non-elongating semi-dwarf						
IR42	18.4 fg	16.6 d	21.3 c	17.9 de	10.8 hi	3.7 ab
BKNFR76106-16-0-1	7.9 h	7.5 c	9.2 d	11.4 e	7.7 f	2.8 b
Non-elongating tall						
NDGR207	34.5 bc	25.1 c	27.7 c	32.8 c	19.0 efg	4.0 ab
Bhatin	32.6 cd	40.7 a	38.3 b	35.9 bc	32.3 bc	4.5 ab
IR28273-R-R-R-29-38-2-3-3	13.9 gh	8.2 e	13.5 d	12.0 e	18.0 fgh	7.9 ab
Elongating modern variety						
IR11141-6-1-4	25.9 de	23.9 c	24.8 c	18.1 de	23.2 def	5.4 ab
IR11288-B-B-69-1	21.4 ef	19.7 de	28.7 c	15.1 e	15.4 gh	9.1 ab
Elongating tall						
IR40905-11-3-1-2-3-2	19.9 efg	22.5 cd	28.0 c	24.7 d	17.8 fgh	9.9 b
Leb Mue Nhang 111	48.0 a	44.8 a	48.4 a	37.4 bc	25.8 cde	9.4 ab
Floating						
Baisbish	38.6 bc	41.3 a	41.8 a	50.3 a	35.6 ab	2.9 ab
Barogar	40.5 b	33.9 b	45.8 b	41.8 b	29.8 bcd	9.1 ab
Kalaungi	37.3 bc	32.5 b	51.5 a	42.1 b	41.8 a	10.9 a
Depth mean	28.2	26.4	31.5	28.3	23.1	6.1
F value	140.3	237.5	154.8	143.5	122.7	

* In a columm, means followed by a common letter are not significantly different at the 5% level.

submergence. Plant elongation was computed by subtracting the plant height before flooding from that after the short-duration flooding treatment. In the control treatment, increase in plant height was calculated by subtracting plant height at 21 days from the height attained by plants at 28 days without flooding.

Appropriate Seedling Age for Assessing Elongation in Some Rice Varieties

Experiment 2: Three seedling ages (2, 3 and 4 weeks) with 9, 21 and 21 entries respectively (Table 2) were laid out in a completely randomised design with three replications to assess variation in plant elongation ability induced by flooding and to select the most appropriate seedling age to be used for further work in the genetics of elongation ability. Seedlings of all ages were submerged for 7 days in 100-cm water depth in the submergence tanks at IRRI.

TABLE 2
Plant elongation (increase in plant height) at various seedling ages following submergence for seven days

Variety	Plant elongation (cm) at seedling ages			Elongation score from previous tests (SES)*
	2 wk	3wk	4 wk	
Group A (Elongating traditional type)				
Saingar	–	37	13	1
Barogar	–	37	20	1
LMN 111	–	42	21	1
Jalmagna	24	27	36	1
NDGR407	25	–	–	1
Chakia-59	–	34	16	3
IR40905-11-3-1-5-3-3	–	25	16	3
NC492	–	28	20	3
Baisbish	26	23	21	3
NDGR150	22	22	7	5
FRG15	–	24	9	5
Madhukar	–	20	105	
NDGR207	15	21	12	5
Group B (Elongating modern type)				
IR11141-6-1-4	17	16	12	5
IR282773-R-R-R-39-28	-	17	18	5
IR11288-B-B-69-1	13	12	11	5
Group C (Non-elongating type)				
Ghoghari	–	18	10	7
Shayma	–	15	9	7
IR42 (Susceptible check)	14	9	8	9
FR13A	–	13	5	9
BKNFR76106-16-0-1	–	8	5	9
IR36	11	9	7	9
CV (%)	9.2	17.6	25.0	

* Standard Evaluation System (IRRI, 1988)

Plant height was recorded before and after the flooding, and was used to calculate plant elongation as adapted in the previous experiment.

Effect of Day Length on Plant and Internode Elongation in Three Deepwater Rice Varieties

Experiment 3: Three varieties, Jalmagna (floating, photoperiod sensitive), IR11141-6-1-4 (deepwater rice, photoperiod insensitive) and RD19 (deepwater rice, weakly photoperiod sensitive) were studied for their response to different day lengths. The pot experiment was laid out in a split plot design with three replications at the Plant Physiology Greenhouse, IRRI. Seedlings at the two-leaf stage were subjected to various periods of 14-hour and 10-hour day length. After 28 days of day length treatment seedlings were submerged in 100-cm water depth for 7 days. Plant elongation and internode length were recorded on 15 randomly selected plants. Two to four plants were examined for panicle primordium initiation before and after the flooding treatment.

RESULTS AND DISCUSSION

Optimum Water Depth

Varieties differed for plant elongation after 7 days' flooding at various water depths (Table 1). Plant elongation rate was higher in Kalaungi, Baisbish and Leb Mue Nahung 111 in almost all depths followed by Barogar and Bhatin. Comparatively less increase was recorded at 80-cm water depth. This may be ascribed to insufficient water depth for full potential elongation as the canopy of most plants emerged above the water during flooding. 11288-B-B-5q-1 and IR11141-6-1-4 (elongating modern varieties) and NDGR207 (non-elongating, tall) were at par showing moderate elongation. Other entries like IR28273-R-R-R-29-38-2-3-3 and IR11288-B-B-69-1 showed relatively less elongation, probably due to their poor seedling growth at the time of flooding. Lowest increase in plant height was recorded in BKNFR76106-16-0-1 and IR42.

By and large, relatively more elongation was recorded at 90 and 100 cm water depth in all floating and deepwater rice varieties. The slight decline in plant elongation at 110 and 120 cm water depths could be due to the inability of plants to emerge above such water depths. Table 2 shows the F values computed for different depths were 140.3 (for 80 cm water depth), 237.5 (90 cm), 159.2 (100 cm), 143.5 (110 cm), and 122.7 (120 cm). Comparatively higher F values for greater elongation at 90 and 100 cm depths support the above findings. This is in agreement with the findings of HilleRisLambers *et al.* (1988) who first used it for comparing various testing methods.

Greater water depths restrict the plant's ability to elongate. As a result there was no clear-cut trend among different varieties at increased water depths. For the purpose of genetic studies, 90-100 cm water depth for seven days might be sufficient to test elongation ability at the early seedling age because using these depths floating types can easily be distinguished from non-floating ones.

Appropriate Seedling Age

Plants stayed alive at all three ages and could be measured individually after flooding. Among the three ages of seedlings, percentage of increase after flooding was highest in the 2-week-old followed by the 3-week-old and 4-week-old seedlings. Entries differed significantly for plant elongation. The comparatively lower elongation in the 4-week-old seedlings might be due to their being taller than the younger ones and thus needing comparatively less elongation for survival.

TABLE 3
Analysis of variance for percent elongation in 12 varieties at 5 water depths and control

Source	D.F.	M.S.
Replications	2	32.0^{ns}
Water depths (d)	5	3013.1**
Error (a)	10	18.6
Varieties (v)	11	1861.0**
Depths × Varieties (d × v)	55	113.9**
Error (b)	132	17.8

CV (a) = 18.0%, CV (b) = 17.6%
** = Significant at 1% level; ns = Not significant.

Table 3 shows that 3-week-old seedlings gave better contrast between elongating and non-elongating modern varieties. If the more minute differences between these two type are to be detected, 4-week-old seedlings should be used. BKNFR76106-16-0-1 and NDGR207, both non-elongating entries, were likewise best separated from the elongating deepwater types in the 4-week treatment.

Day Length Effect and Plant Elongation

Table 4 shows significant differences among the treatments for plant elongation and internode length. Greatest plant elongation was recorded for Jalmagna followed by RD19 and IR11141-6-1-4. The trend for internode length was similar. In addition, varieties × treatment interaction was also found to be significant for internode length after flooding treatment. Invariably all three varieties had maximum internode length at short day length while shortest internode length was obtained with long day treatment.

None of the varieties initiated panicle primordia in any of the treatments even up to 7 days after flooding treatment. Therefore, internode elongation may only be due to the signal effect of short day length or the shading effect on assimilation as other factors were kept constant.

The effect of day length treatment was significant in the case of the two elongation modern varieties but not in the case for traditional Jalmagna. This may be due to the intrinsic tall plant height of the latter, which prevented it from responding strongly to flooding. The fact that there was no gradual transition from long internodes to short internodes with decreasing duration of short day treatment favours the explanation that internode elongation is a signal function of short days.

CONCLUSION

Experiments conducted with different entries to study the effect of water depth, seedling age and day length on elongation

TABLE 4
Treatment and variety means for plant elongation and internode length of three rice varieties.

Days subject to treatment		Jalmagna		IR11141-6-1-4		RD 19	
10h	14h	Pl. elongation (cm)	Internode (cm)	Pl. elongation (cm)	Internode (cm)	Pl. elongation (cm)	Internode (cm)
28	0	57.2	83.8	33.6	25.4	44.2	37.0
21	7	64.1	79.2	29.4	25.4	42.8	36.2
14	14	61.5	78.1	30.4	25.1	32.4	28.3
0	28	64.5	77.3	38.7	15.4	36.7	1.4

	Plant elongation	Internode length
S.E.D.	4.2	2.2
CV (%)	11.8	6.9

resulting from a short period of submergence (7 days) indicate that three-week-old seedlings with 90-100 cm water depth are most effective for assessing elongation ability at the seedling stage. The test was non-lethal. Results further indicated that short day treatment induced maximum plant elongation in some varieties.

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Response of French Bean (*Phaseolus vulgaris* L.) to Rate and Ratio of Potassium Fertilizer Application

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Keywords: French bean, potassium, growth, yields

ABSTRAK

Kajian lapangan telah dijalankan untuk menilai kesan baja potasium, apabila digunakan pada nisbah yang berbeza sebagai baja pangkal atau permukaan terhadap pertumbuhan vegetatif, lenggai tak matang dan hasil biji benih kacang peranchis. Begitu juga kualiti biji benih untuk tujuan penanaman yang ditentukan oleh percambahan sebelum atau selepas kekurangan nilai dikawal. Kadar penggunaan baja pada kadar (100:0, 50: atau 0:100 telah ditambah semasa penanaman (pangkal) atau pada pembungaan (peringkat R 1-pembajaan permukaan). Pertumbuhan kacang perancis tidak dipengaruhi oleh potasium. Perbezaannya, pertumbuhan vegetatif dan komponen paling berhasil jelas meningkat sehingga 100 kg K_2O per hektar. Penggunaan kadar yang diberikan hanya pada penanaman (100:0) meningkatkan pertumbuhan vegetatif dibandingkan dengan bila kadar itu diasingkan. Penggunaan pada pembungaan semata-semata (0:100) mengurangkan semua parameter terukur, menentukan keperluan potasium sewaktu penanaman. Hasil lenggai tak matang yang dituai untuk tujuan penanaman sayur nyata tidak bertambah oleh dua penggunaan dalam nisbah 50:50 (pangkal: pembajaan permukaan). Kualiti biji benih ditentukan oleh percambahan sebelum dan selepas kekurangan nilai dikawal juga terlibat dan penggunaan berasingan 100 kg K_2O dalam nisbah 50:50 meningkatkan percambahan.

ABSTRACT

A field study was undertaken to evaluate the effect of potassium fertilizer, when applied in different ratios, as a basal or top dressing on vegetative growth, immature pod and seed yield of French bean as well as seed quality for planting purpose determined by germination before and after controlled deterioration. The ratios of fertilizer application at a given rate of 100:0, 50: or 0:100, were added at planting (basal) or at flowering (R1 stage - top dressing). Establishment of French bean was not affected by potassium. In contrast, vegetative growth and most yield components increased significantly with up to 100 kg K_2O per ha. Application of a given rate only at planting (100:0) enhanced vegetative growth compared with a split rate. Application at flowering alone (0:100) reduced all measured parameters, confirming the requirement of potassium at planting. Yields of immature pods harvested for vegetable purposes were not significantly increased by the two applications in the ratio of 50:50 (basal: top dressing). Seed quality determined by germination before and after controlled deterioration was also affected and split applications of 100 kg K_2O in the ratio of 50:50 increased germinability.

INTRODUCTION

Piggot (1986) illustrated a greater requirement of potassium at flowering in French bean to facilitate the heavy sink effect by developing pods and seeds, and the role of this nutrient in the translocation of photosynthates. Thus general application at planting alone can lead to deficiencies, especially when the crop is grown for seed over a long period of time (Tindall 1983; Adams *et al.* 1985). Thung (1991) suggested that potassium would become a limiting factor for successful production of French bean, due to high rates of utilization and depletion of this element. Thus a study was conducted to identify the impact of differ-

ent rates of potassium application on yields of immature pods and mature seed of French bean and to determine the effects of applying selected rates either at planting (basal), at planting and flower initiation (basal and top dressing) or at flowering alone (top dressing) on growth and yields of immature pods and mature seeds. The impact of these treatments on the quality of seeds for planting material was also determined by controlled deterioration, as farmers generally cultivate this crop from seeds of previous seasons.

MATERIALS AND METHODS

The experiment was carried out at the experimental unit of the Faculty of Agriculture, University of Peradeniya, Sri Lanka (7^0N, 81^0E, 470 m above sea-level), which has a tropical south Asian monsoonal climate (Domros 1974), with a mean annual rainfall of 1700 mm spread over two seasons (the rainfall over the experimental period beginning November, 1989 was 548 mm) with a mean monthly temperature of 27.4 ± 1.24^0C, and humidity of $75.2 \pm 2.32\%$. The daylength was 11-12 hours. The soil at the site is classified as an Alfisol, with a sandy clay loam texture. The important characteristics of the top 30 cm were as follows:- pH(1.25 H_2O) 6.54 ± 0.31, organic C (Walkley and Black) 0.66% (w/w), Total N (kjeldahl) 0.42%, exchangeable K 121 ppm and a CEC of 42.2 m eq per 100 g of soil.

Uniform seeds (germination 95.2%) of the bushy type of French bean (variety Wade) were planted in well-prepared 2×3 m seed beds, at a spacing of 20×10 cm. The crop was managed as per recommendations of the Department of Agriculture (1988) and was manually weeded on two occasions. No supplementary irrigation was required.

The fertilizer treatments adopted were equivalent to 0, 50, 100 or 150 kg K_2O per ha, supplied in the form of KCl (60% K_2O). Uniform rates of 50 kg P_2O_5 and 30 kg N were applied at planting. The selected potassium levels were applied in the following ratios at planting (basal) or at flower initiation (R1) (Fageira *et al.* 1991): (A) 100:0 (B) 50:50 and (C) 0:100. At the time of the top dressing of potassium, nitrogen equivalent to 15 kg N per ha was applied, irrespective of application of potassium, to maintain uniformity. The experiment was laid out as a randomized block design with four replicates, with fertilizer treatments randomized within the block.

Crop establishment (percentage of planted seed) was determined at 8 days after planting. Thereafter, four plants were removed from each plot at 6-day intervals up to flower initiation (R1) and dry weights determined by desiccation at 80^0C for 48 hours. These data were used to calculate relative growth rates (g/g/wk). The ratio of RGR of fertilized plants to that of the control ($0K_2O$) was computed to determine the treatment effect on vegetative growth.

At 50% flowering (growth stage R3), 30 plants within each plot were tagged to determine flower and pod numbers and seeds per pod. At the time of immature pod harvest (R7), 15 of the tagged plants were used to determine yields per plant and weight of immature pods. The other 15 plants were harvested at full maturity (R8) when most mature pods had begun to split, and seed yield per plant and 100-seed weight were obtained.

The controlled deterioration tests adopted were similar to those described by Matthews and Powell (1987) and Hampton *et al.* (1992). Thus, four replicates, each containing 40 g of seeds obtained from the different potassium treatments were soaked in deionized water to obtain a 24% seed moisture content. The quantity of water added was determined by the equation

described by Hampton *et al.* (1992). Thereafter, the seed samples were sealed in aluminium foil and incubated for 72 hours at 45^0C. Germination of seeds before and after controlled deterioration was evaluated by counting the number of normal seedlings at 14 days after planting to a depth of 2.5 cm in sand. Statistical analysis was carried out by ANOVA and comparisons by LSD, as described by Gomez and Gomez (1981).

RESULTS AND DISCUSSION

Vegetative Growth

Rates and ratios of potassium fertilizer application had no impact on the establishment of French bean (Table 1). In contrast, the ratios of relative growth rates (RGR) illustrate the benefits of applying potassium in enhancing the development of the emerged seedling. The RGR of plants in the control (0 K_2O) plots was 0.043 ($\pm$0.002) g/g/wk, which was considered the baseline. The RGR ratio increased significantly with increasing rates of K_2O, although the increment with 150 kg K_2O was marginal over that of 100 kg K_2O. Thus 100 kg K_2O could be considered optimal for good vegetative growth of French bean under the conditions of this trial.

Application of potassium levels at planting (100:0) developed the highest RGR, while the supply of 50% of a given rate at planting depressed growth. The absence of K_2O at planting showed growth rates similar to those of the control. This clearly illustrates the requirement of potassium in the basal fertilizer, as the element influences the vegetative growth phase of the plant (Wolley *et al.* 1991). However, at higher rates of K_2O (i.e. 150 kg), application of 50% of potassium at planting does not increase the RGR ratio significantly over that of the 100:0 (basal: top dressing)

TABLE 1
Effect of rate and ratios of potassium fertilizer application on vegetative growth of French bean

Rate (kg K_2O) per ha	Ratio*	Establishment (%)	RGR Ratio**	Days to Flower Initiation
0	–	76	1.0	39
50	A	87 a+	1.23 a	34 a
	B	93 a	1.08 b	37 bc
	C	95 a	1.02 c	39 c
100	A	89 a	1.42 a	31 a
	B	94 a	1.25 b	35 b
	C	96 a	0.95 c	40 c
150	A	95 a	1.46 a	30 a
	B	91 a	1.34 b	33 b
	C	95 a	1.06 c	39 c
LSD P=0.05 (Means)		5.02	0.024	2.11
Interaction		NS	*	*

* Ratio of application – A = 100:0, B = 50:50, C = 0:100 (Basal: top dressing) at planting and flowering

** RGR ratio = $\frac{\text{RGR of treatment}}{\text{RGR of control (0 } K_2O)}$ (units of RGR g/g/wk)

+ In a coloumn, means within a given role of fertilizer followed by a common letter are not significantly different (p = 0.05)

ratio of the same quantity. This is due to adequate supply of the element for early growth by this ratio at this rate.

Flowering

Application of potassium at ratios of 100:0 or 50:50 (basal: top dressing) reduces the time to flower initiation in French bean (Table 1), and lack of K_2O at planting delays this process, which could be attributed to the weaker growth of the plants shown by RGR ratios. Number of flowers on a plant is significantly increased by application of 50 or 100 kg K_2O at planting, due to better vegetative growth at these rates and ratios. This may be attributed to reduced photosynthetic processes and carbohydrate metabolism (Ting 1982; Fageira *et al.* 1991), which influence the initiation of the reproductive phase in plants.

Pod and Seed Numbers

A similar phenomenon is observed on pod number (Table 2), although potassium fertilizer probably does not have an impact on the process of pollination. However, flower abortion could be enhanced in conditions of potassium deficiency in legumes (Hanway and Johnson 1985), which could influence the number of pods per plant. At the lower rates of K_2O, split application (50:50 basal : top dressing) reduced number of flowers and pods per plant. This was not seen at the highest rate, due to the application of sufficient quantities at planting. At all rates, application of all potassium at flowering (0:100) reduced flower and pod number, due to poor early growth of the plant. The importance of potassium in enhancing reproductive growth in French bean (Fageira *et al.* 1991) could also have affected pod growth.

Seed number per pod (Table 2) was not influenced by the rates and ratios of potassium application, except in the control treatment where the reduction was marginal. However, pod and seed development were influenced by the rates and ratios of potassium. The absence of potassium in the basal fertilizer reduces both pod and seed weights significantly. This confirmed the importance of potassium throughout the growth of the crop.

TABLE 2
Effect of rate and ratio of potassium application on yield components of French bean

Rate (kg K_2O/ha)	Ratio*	Flowers/ Plant	Pods/Plant	Seeds/Pod (g)	Wt of Pod (g)	100-seed Wt
0	–	6.2	4.0	4.5	4.9	17.42
50	A	16.2 a+	12.5 a	5.2 a	7.1 a	23.46 a
	B	10.3 b	8.8 b	5.4 a	7.3 a	24.15 b
	C	6.5 c	4.5 c	5.0 a	5.2 b	19.15 c
100	A	24.6 a	20.6 a	5.2 a	8.6 a	25.44 a
	B	20.2 b	17.5 a	5.6 a	8.9 a	28.40 b
	C	6.9 c	4.8 b	5.1 a	6.1 b	22.16 c
150	A	26.5 a	20.5 a	5.2 a	8.7 a	26.24 a
	B	25.4 a	20.1 a	5.3 a	9.2 a	30.65 b
	C	8.5 c	5.9 b	4.9 a	6.2 b	22.68 c
LSD P = 0.05 (Means)		3.9	4.81	1.96	0.76	4.09
Interaction		*	*	NS	*	*

* Ratio of applications – A = 100:0, B = 50:50, C = 0:100 (Basal : top dressing) at planting and flowering

\+ In a column, means within a given rate of fertilizer followed by a common letter are not significantly different (p = 0.05)

Pod and Seed Yields

Application of potassium only on planting also reduced pod weight marginally, and seed weight significantly as both these are important sinks and the supply of carbohydrates is influenced by potassium (Mengel and Kirby 1987). Thus, application of potassium at planting and flowering ensures an adequate supply for plant growth, thereby increasing seed weight significantly. The greater impact of spilt application on seed weight could also be attributed to the longer period taken for seed maturity, as compared with that of immature pods harvested as a vegetable. Application of low rates of potassium at planting alone may not meet the demand of the crop during seed development due to the high solubility of this element, which leads to leaching losses, especially under the rainfed conditions which prevailed during this study.

Application of potassium increased both pod and seed yield of French bean (Table 3), due to better vegetative growth and enhanced values of yield components. Although yields are further increased with 150 kg K_2O the increment between 100 and 150 kg is marginal. Thus, in this study, application of 100 kg K_2O per ha is considered the optimal rate, which corresponds to most potassium fertilizer recommendations for French bean in the humid tropics (Tindall 1983; Thung 1991).

Withholding potassium in the vegetative phase reduces both pod and seed yields significantly. This confirms the importance of potassium in basal fertilizers for French bean, as for other legumes (Hanway and Johnson 1985). However, immature pod yields are not significantly increased by applying potassium at planting and flowering, especially at 100 or 150 kg K_2O per ha. At 50 kg K_2O per ha, there is a significant increment in pod yield when all of the potassium is applied as a basal dressing. This could be attributed to the influence of potassium on the vegetative growth, as a split application of 50 kg K_2O may not provide adequate quantities for optimal growth rates (Table 1). Thus farmers growing this crop for vegetative purposes can obtain high yields with one application of potassium at planting.

TABLE 3
Effect of rate and ratio of potassium fertilizer application on fresh pod and seed yield of french beans

Rate (kg K_2O/ha)	Ratio*	Pod Yield$^+$ (g/plant)	Seed Yield** (g/plant)
0	–	20.4	4.82
50	A	69.6	14.46
	B	66.5	15.84
	C	28.6	4.96
100	A	138.1	23.57
	B	146.6	27.94
	C	38.4	6.95
150	A	153.7	25.82
	B	159.9	31.95
	C	68.3	9.96
LSD (P = 0.05) within a rate of K_2O		9.45	2.61
Means of rates		1.96	0.96
Interaction		*	*

* Ratio of applications – A = 100:0, B = 50:50, C = 0:100 (Basal : top dressing) at planting and flowering
\+ Pod yield determined by the harvest of fresh immature pods for vegetable purposes
** Seed yield corrected to 20% moisture at harvest

Application of potassium only at planting reduced seed yield significantly. Thus split applications are required, except at the lowest rate (50 kg K_2O per ha). In contrast to pod production, optimal seed yields are obtained by split applications, although most farmers cultivating this crop apply fertilizers once at planting (Thung 1991). The longer period of growth required for seed production needs a split applications of this soluble nutrient. The study also illustrates that applications of 100 kg K_2O as a split application provides similar yields to those of applying 150 kg K_2O at planting. Thus, judicious applications of potassium also reduces the quantity required.

Seed Quality

Potassium fertilizer influences seed quality of French bean due to its role in plant metabolism (Fageira *et al.* 1991). Thus seed germination is increased significantly by the addition of 100 kg K_2O, irrespective of the ratio of the application (Table 4), as are pod and seed yields, for which this is the optimal rate (Table 3). As for seed yields, application of potassium at flowering reduces germination, both before and after controlled deterioration. Thus, for optimal seed quality, potassium is required from planting. A comparison of germination values again indicates the importance of split applications of potassium in increasing seed quality for planting purposes, as measured by germination before and after controlled deterioration. The patterns of germination due to potassium are not altered by controlled deterioration. This is clearly evident at 100 and 150 kg K_2O, while the effect at 50 kg K_2O is marginal. This illustrates that potassium is required at both the vegetative and reproductive phases for high yields of good quality seed, which can be used for immediate consumption or planting at a later season, the latter being more important in the developing world of Asia.

TABLE 4
Effect of rate and ratio of application of potassium fertilizer on germination pattern of French bean after controlled deterioration

Rate of K_2O (kg/ha)	Ratio*	Germination (%)	
		before cd	after 72 hrs cd
0	–	42	18
50	A	57	31
	B	59	39
	C	47	24
100	A	74	52
	B	91	72
	C	51	31
150	A	81	54
	B	94	67
	C	53	36
LSD (P = 0.05) Within a rate of K_2O		6.01	4.90
Means of rates		3.84	2.37
Interaction		*	*

* Ratio of applications - A = 100:0, B = 50:50, C = 0:100 (Basal : top dressing) at planting and flowering
\+ Germination determined by number of emerged normal seedlongs in 7 days

CONCLUSIONS

Potassium is a prerequisite for optimal yields of legumes, including French bean as this field study clearly illustrates. Both rates and ratios of potassium application influence vegetative growth, pod and seed yields and the quality of seeds, measured in terms of germinability. Farmers producing immature pods for vegetable purposes should apply potassium at planting to obtain high yields. In contrast, for the production of high seed yields (for both consumption and planting) applications at planting and at flowering are required. This will ensure good seed quality determined by controlled deterioration, for planting purposes. Split applications can reduce the requirement of fertilizer potassium.

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Differences in Functional Properties of Mungbean Protein Concentrate and the Effect of Incorporation into Fish Sausages

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ABSTRAK

Sifat fisiko-kimia dan sifat berfungsi pekatan protein kacang hijau yang dihasilkan melalui (i) mendakan menggunakan kalsium sulfat dan (ii) mendakan menggunakan titik isoelektrik, bila dibandingkan, mengandungi 21.6 dan 67.3% protein masing-masing. Kelarutan pekatan pekatan tersebut berkait secara positif dengan pH dalam banjaran nilai pH 4-7. Kebolehan membusar berkait rapat dengan percentage of N larut ($r^2 = 0.98$) dan pH ($r^2 = 0.88$) sementara kestabilan busar ada kaitan dengan kehidrofilikan ($r^2 = 0.98$) pekatan. Semua pekatan dapat mengurangkan kehilangan berat, kekecutan dan meningkatkan ketegasan sosej ikan yang telah di masak. Peratus kehilangan berat dan kekecutan berkait secara negatif dengan peratus protein larut, pH dan sifat kebolehan membusar pekatan. Sifat-sifat berfungsi pekatan-pekatan tersebut, bila ditambah kedalam sosej ikan pada kandungan 1-2% akan mempengaruhi tekstur produk tersebut. Dalam penilaian deria, sosej ikan yang mengandungi protein-protein tumbuhan mendapat skor yang lebih tinggi untuk penerimaan keseluruhan, walaupun tiada perbezaan bererti dalam skor cita-rasa atau tekstur dan skor yang lebih rendah didapati untuk kesarian produk bila dibandingkan dengan hasil kawalan.

ABSTRACT

The physico-chemical and functional properties of mungbean protein concentrate prepared by (i) calcium sulphate precipitation (MBC-Ca) and (ii) isoelectric point precipitation (MBC-pI) containing 21.6 and 67.3% protein respectively, were compared. The solubility of the concentrates was positively correlated with pH within the range of 4-7. The foaming ability was closely correlated with percentage of soluble N ($r^2 = 0.98$) and pH ($r^2 = 0.88$) while the foam stability was correlated with the hydrophilicity ($r^2 = 0.98$) of the concentrates. All concentrates were able to reduce the weight loss, shrinkage and increase the firmness of cooked fish sausages. The weight loss and shrinkage were negatively correlated with the soluble protein, pH and foaming ability of the concentrates. The functional properties of the concentrates, when added at a level of 1-2%, influenced the texture of the fish sausages. In organoleptic evaluations, fish sausages incorporating the plant proteins scored higher for overall acceptability, even though there was no significant difference in flavour or texture and a decrease in juiciness of the product compared to the control.

INTRODUCTION

Mungbean (*Phaseolus aureus*) contains about 20-27% protein and has an amino acid profile comparable to soybean (Evans and Bandermer 1967; Fan and Sosulski 1974; Thompson *et al.* 1976). It is an important protein source in India, and partially replaces some of the ingredients for baby foods, snacks and noodles in the Philippines, China and Japan (Bhumiratana and Nondasuta 1972). However, its colour and 'beany' flavour limit its use, unless it is dehulled or converted to protein concentrate. Mungbean protein concentrate (MBC) can be a by-product of mungbean noodle factories, which only make use of the starch. MBC may not only improve the nutritional content but also the flavour, texture and appearance of the food.

The quality of MBC has been shown to be comparable to soy protein concentrate (SPC) (Thompson 1975; Bhumiratana 1977). Studies on MBC have been limited to its use as a meat analogue (Narayana and Narasinga Rao 1982). Its functional properties have not been extensively studied with regard to its usefulness as a stabilizer, thickener, milk substitute, emulsifier, extender and binder in various products. The functional properties of MBC merit research for developing its use in food, especially in cereal-based products because of the complimentary amino acid pattern. This work was carried out to compare selected functional properties of MBC prepared by two different methods and the effect of incorporating it in a product such as fish sausage.

MATERIALS AND METHODS

Dehulled mungbeans from Thailand of unknown storage history were obtained from retail shops near the university. Foreign particles and spoiled beans were removed. Mungbean flour (MBF) was prepared by grinding the beans in a hammer mill to pass 710 nm mesh size sieve (U.S. standard mesh). Commercial grade soya protein concentrate from defatted soya flour (Marksaids Malaysia) was used for comparison (standard) of the functional properties of prepared mung bean concentrate (MBC). Fresh bighead carp (*Aristichthys nobilis*), obtained from Salak South, Kuala Lumpur, were harvested at 6-9 months maturity (about 45-55 cm long and 1.8-2.5 kg weight). Unless otherwise stated, all experiments were carried out at room temperature (30 ± 3°C). Preparation of MBC was carried out in 6 replicates.

Calcium-precipitated MBC (MBC-Ca) was prepared by extracting MBF with about 5 times its weight of water and filtering through a muslin cloth. The extracts were brought to 90°C, cooled to 85°C, allowed to precipitate for about 30 min with 0.4% (w/w) $CaSO_4$ (BDH Chemical Ltd, Poole, England), centrifuged and dried at 45°C for 24 h before grinding and sieving (280nm sieve) (Payuma *et al.* 1985).

Isoelectric point-precipitated MBC (MBC-pI) was precipitated from similar aqueous extracts of MBF by adjusting the pH down to the isoelectric point of the mungbean protein (pH 4.0) with 6N HCl (Analytical grade, Gruppo Montedison, Farmatalia CaHo Erba), centrifuging and drying as above (Chang and Satterlee 1979).

The percentage of soluble nitrogen of the protein was determined by the Biuret method using bovine serum albumin (Sigma, B2518 lot No33H6780) as the standard (Layne 1957; Narayana and Narasinga Rao 1982). Emulsifying ability was determined by dropping RBD palm olein at 0.2 ml/s into a continuously stirred (Magnetic stirrer) suspension of 2.0 g MBC in 23 ml distilled water (Lin *et al.* 1974). The end point was when the ammeter needle (Sanwa, YX-360 TR, Taiwan) suddenly showed a change in reading and the emulsion separated into two phases.

The foaming ability (Lawhon *et al.* 1972) was the foam volume at 30 s after homogenizing 100 ml, 1.0% aqueous suspension of MBC for 5 min using a Kinematica emulsifier (Switzerland) as measured using a 250-ml measuring cylinder. The volume of the foam was monitored every 5 min for 120 min. The foam stability was calculated by the formula $(2t/50V_m)$ where V_m = max foam volume (ml), and t = time in min for the foam to collapse to $V_m/2$ (Townsend and Nakai 1983).

Fat-holding capacity (Lin *et al.* 1974) was determined by measuring the free oil remaining after mixing 0.5g MBC to 5.0 ml of RBD palm olein for 30 s, standing for a further 30 min, and then centrifuging for 25

min at 1750 g. Colour was determined using the Hunter-lab colorimeter (model D25, USA) with a white tile (a = -0.9, b = 0.5 and L = 91.25) as the standard. pH was measured using a pH meter (Jenway, Model PHM64). Moisture was determined by drying 2.0 g MBC *et* 105°C to constant weight (AOAC 1980). Crude protein was determined on 0.15 g MBC using the micro-Kjeldahl method where crude protein = N × 6.25 (AOAC 1980).

Preparation of Fish Sausage

Fish sausage was prepared by mixing 50g minced fish flesh, 50g tapioca flour and 1g salt. The proximate composition of the fish sausage was 16.4% protein, 4.3% fat, 76.9% moisture and 1.2% ash. Protein concentrate was added to minced fish meat at 0, 1.0, 1.5 and 2.0% level in the presence of 1% NaCl. They were mixed in a Kenwood Chef mixer (model A901) at a speed of 4 rev/s for 5 min at room temperature, then inserted into 35-mm diameter cellulose casing and tied at the ends. After labelling, the sausages were kept frozen (at -20°C) for 24 h to allow for the formation of intermolecular crosslinks.

Analysis of the Fish Sausage

Frozen fish sausage was thawed at 30°C for 30 min, boiled for 25 min, then air cooled for 15 min.

The weight loss and shrinkage were determined by comparing the average weight and circumference of six sausages before and after boiling.

The texture of the sausage was empirically determined using an Instron Universal testing machine (Model 1140) with a 5-cm long puncture probe on thawed sausage samples at a crosshead speed of 50 mm/min, a 5 kg load cell and noting the yield/breaking stress.

The cooked fish sausages were organoleptically evaluated by 10 trained panellists for colour, flavour, texture, juiciness and overall acceptability on a 1-7 hedonic scale (7 = extremely like; 1 = extremely dislike) (Larmond 1982). The data were analysed using multiple-range test (Walpole 1982) and regression analysis.

RESULTS AND DISCUSSION

The protein content of both MBCs was found to be lower than that of SPC (Table 1). The solubility of the protein was positively correlated ($r^2 = 0.83$) with pH within the range studied independently of how they were prepared. Therefore, theoretically the solubility could be adjusted as required by bringing the pH away from the isoelectric point of the protein and increasing the net charge.

Regression analysis shows that the amounts of moisture in the MBCs and SPC after preparation were positively correlated with the percentage of soluble crude protein of the concentrates ($r^2 = 0.99$), indicating that the hydrophilic nature of the soluble protein has some influence on the moisture content of the concentrates.

Emulsifying Capacity and Fat Absorption Capacity

For all concentrates, the values of fat absorption capacity and emulsifying capacity followed a similar trend; the highest value was seen in MBC-pI and the lowest value in MBC-Ca (Table 1). Voutsinas and Nakai (1983) showed that a close relationship exists between fat binding capacity and surface hydrophobicity, while Kato and Nakai (1980) found a significant correlation ($P < 0.01$) between the emulsifying capacity and the hydrophobicity of proteins. The present study confirms the close relationship between fat-binding capacity and emulsion capacity, and that they most likely relate to the surface hydrophobicity of the protein molecules. The result above thus indicates that the

TABLE 1
Physico-chemical properties of calcium sulphate precipitated mungbean protein concentrate [MBC-Ca], isoelectric point precipitated mungbean protein concentrate [MBC-pI] and soya protein concentrate [SPC]

Sample	MBC-Ca	MBC-pI	SPC	MBF (mungbean flour)
crude protein (%)	$51.6^{c} \pm 0.6$	$67.3^{b} \pm 4.1$	$77.1^{a} \pm 2.2$	$22.3^{d} \pm 1.6$
moisture (%)	$10.35^{b} \pm 10.05$	$10.18^{b} \pm 0.14$	$12.08^{a} \pm 0.05$	
soluble N* (%)	$2.6^{c} \pm 0.7$	$2.1^{c} \pm 0.5$	$6.3^{a} \pm 0.4$	$3.6^{b} \pm 0.3$
pH	$5.71^{b} \pm 0.01$	$4.06^{c} \pm 0.04$	$7.01^{a} \pm 0.003$	6.25
fat absorption (g oil/g protein)	$1.9^{a} \pm 0.3$	$2.4^{a} \pm 0.7$	$2.3^{a} \pm 0.6$	
emulsifying capacity (g oil/g protein)	$15.2^{c} \pm 0.6$	$30.2^{a} \pm 10.8$	$21.7^{b} \pm 0.4$	
foaming ability (ml)				
0.5 min	12.0	3.9	36.0	
5.0 min	7.3	3.9	36.0	
120 min	4.3	0.5	18.7	
Best fitted line				
Y =	6.84–0.016x	3.015–0.023x	36.17–0.145x	
r^2 =	0.74	0.85	0.99	
rate of foam collapse (ml/min)	0.02	0.02	0.15	
foam stability ($2t/50V_m$)	0.17	0.46	0.14	
t = (min) time to reach $V_m/2$	50	45	125	
colour				
(L) lightness	$84.88^{a} \pm 0.05$	$84.40^{b} \pm 0.03$	$82.35^{c} \pm 0.03$	
(a) + red, -green	$-0.72^{a} \pm 0.08$	$-2.15^{b} \pm 0.03$	$-1.99^{c} \pm 0.05$	
(b) + yellow, -blue	$12.77^{a} \pm 0.19$	$18.05^{b} \pm 0.03$	$30.38^{c} \pm 0.11$	

* = pH unadjusted

Means within the same row followed by the same letter are not significantly different ($p > 0.05$). The standard deviations were calculated from at least 6 replicates

surface hydrophobicity of MBC-pI is much greater than of MBC-Ca. At the isoelectric point, the electrostatic charges on the randomly coiled heated protein molecule interact, causing exposure of more hydrophobic side chains (Bigelow 1967) and protein insolubility.

However, there was poor correlation between emulsifying capacity or fat absorption and protein solubility. This agrees with the work of Wang and Kinsella (1976) who found no statistical correlation between emulsifying capacity and protein solubility, but a high correlation ($r^2 = 0.8$) between emulsion stability and protein solubility. The emulsifying properties of proteins ultimately depend on the hydrophile:lipophile balance, and do not necessarily increase as the proteins become more hydrophobic (Rand 1976). The ability of proteins to bind lipids is important for applications such as meat replacers and extenders.

In this study, under the same rates of blending, oil addition and temperature, the fat absorption capacity and emulsion capacity were found to be correlated only with the protein content in the concentrates ($r^2 = 0.87$ and 0.52 respectively) and not with pH.

Foaming Ability

The foaming ability was very closely correlated with the percentage of soluble N ($r^2 = 0.98$), and also with pH of the concentrate ($r^2 = 0.88$), as Cheftel *et al.* (1985) and Townsend and Nakai (1983) respectively had also found. The availability of mixtures of acidic and basic proteins (opposite charges) is thought to be important for the inter-molecular electrostatic interactions and strength of the 'skin' around the air bubbles (Hart 1986).

Foaming ability was found not to be related to emulsion capacity or fat absorption capacity, but surface hydrophobicity, which confirms the work of Townsend and Nakai (1983). The presence of salt in the final product (as with MBC-Ca and MBC-pI preparation) also reduces foam capacity and stability (Graham and Phillips 1976), but increases emulsion capacity (Wang and Kinsella 1976) due to the unfolding of the protein. The salt content or ionic strength of the MBCs was not determined in this work.

Foam Stability

Rate of foam collapse was found to be closely correlated ($r^2 = 0.98$) to the water-holding ability (hydrophilicity) of the concentrates, the soluble nitrogen content ($r^2 = 0.97$) and the foaming ability ($r^2 = 0.92$). Foam stability is positively correlated with emulsion capacity ($r^2 = 0.75$) and negatively correlated with pH ($r^2 = 0.86$) and foaming ability ($r^2 = 0.56$). Hydrophobes are thought to cause foam collapse by competing with protein at the bubble surface, thus disrupting the continuity of the adsorbed protein film (Hart 1986).

Colour of MBC

There is little colour difference between MBC-pI and SPC. The colour was found to be affected by pH, moisture, protein and fat content and soluble N of the concentrates.

Effect of the MBCs on the Properties of Fish Sausage

When the MBCs were added, the properties of the concentrates affected the properties of the fish sausage (*Figs. 1* and *2*). The percentage of protein in the concentrate is positively correlated with the organoleptic texture score ($r^2 = 0.75$). The increase in firmness with the addition of MBCs was preferred by the panellists over the control, while addition of SPC was disliked.

The percentage of soluble nitrogen in the protein concentrates favourably affected the sausage weight loss ($r^2 = 0.78$), shrinkage ($r^2 = 0.71$) and Instron-measured firmness ($r^2 = 0.92$). The fish sausage samples which contained protein concentrates were preferred to the normal fish sausages for flavour and texture.

Increasing the pH of the protein concentrates reduced weight loss ($r^2 = 0.50$), shrinkage ($r^2 = 0.89$) and Instron measured firmness ($r^2 = 0.85$) but decreased the flavour ($r^2 = 0.55$) and sensory scores for juiciness ($r^2 = 0.83$). This can be attributed to the increased net charge on the MBCs with increasing pH

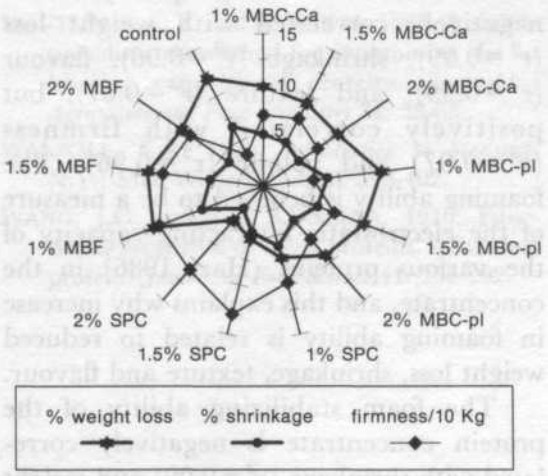

Fig. 1. Effect of protein concentrate on weight loss, shrinkage and firmness of fish sausages

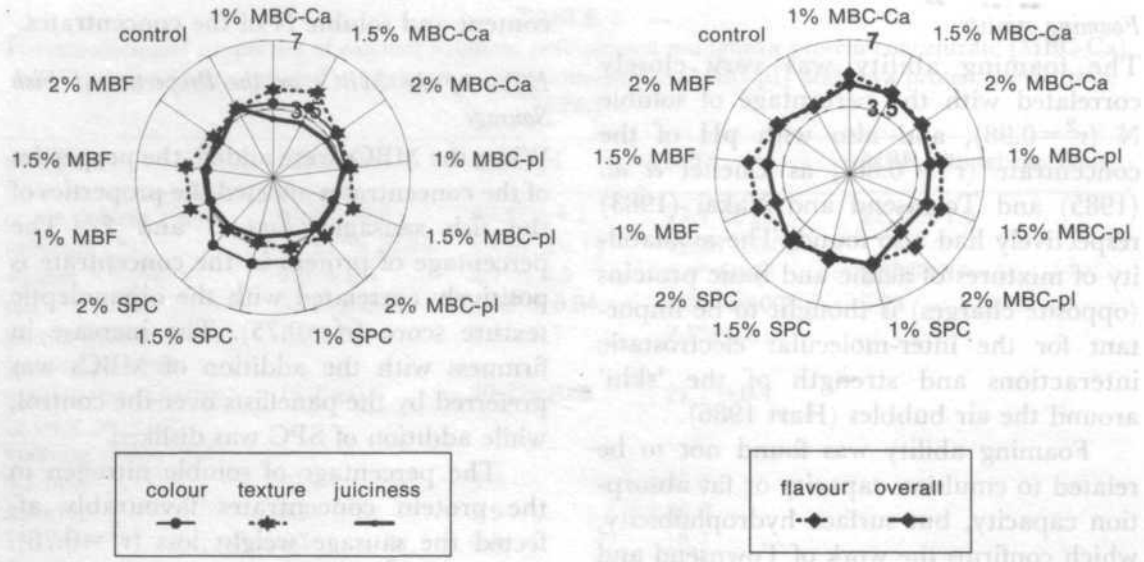

Fig. 2. Sensory evaluation of fish sausages containing plant proteins

above the pI, thus increasing the swelling and the water-binding ability of the MBCs.

The emulsifying capacity and fat absorption capacity do not have much effect on the fish sausage. This functional property may have more influence in meat sausages, which have a greater percentage of fat included in the formulation.

The MBC foaming ability greatly influenced the fish sausage quality, and is negatively correlated with weight loss ($r^2 = 0.99$), shrinkage ($r^2 = 0.96$), flavour ($r^2 = 0.99$), and texture ($r^2 = 0.67$), but positively correlated with firmness ($r^2 = 0.97$) and colour ($r^2 = 0.96$). The foaming ability is believed to be a measure of the electrostatic interacting capacity of the various proteins (Hart 1986) in the concentrate, and this explains why increase in foaming ability is related to reduced weight loss, shrinkage, texture and flavour.

The foam stabilizing ability of the protein concentrate is negatively correlated with shrinkage ($r^2 = 0.99$) and weight loss ($r^2 = 0.56$), and has been shown earlier to be correlated with the hydrophilicity of the concentrate.

In all experiments the weight loss of fish sausage was reduced by adding protein concentrate (*Fig. 1*). The original sausage weights were better retained with SPC > MBC-Ca > MBC-pI > MBF. Weight loss is mainly due to the reduction in water-holding capacity of the heat-denatured protein. Adding the protein concentrates also increased the firmness of the fish sausages in the following order SPC > MBF > MBC-Ca > MBC-pI. Sensory evaluation (*Fig. 2*) showed that the samples of fish sausage with added plant protein were preferred (overall acceptability) to the control sausages. Generally, there was no significant difference in texture and flavour, but a decrease in juiciness was observed in the fish sausages with added plant protein. Colour scores for the sausages was higher with the addition of SPC. The colour scores for sausages with added MBC-Ca were insignificantly different from those of the control, but those with MBF or MBC-pI scored slightly unfavourably.

CONCLUSION

Incorporation of mungbean protein concentrate increased the overall acceptability of fish sausages by reducing the weight loss, shrinkage and increasing their firmness.

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Nutrient Content in Rice Husk Ash of Some Malaysian Rice Varieties

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Keywords: rice husk ash, nutrient, variety, fertilizer

ABSTRAK

Analisis terhadap 60 sampel bijirin mewakili 10 varieti padi menunjukkan 21.33% kandungan sekam dan 13% abu. Dari segi kandungan nutrien abu sekam, 80.26% terdiri dari silika, 0.38% fosforus, 1.28% kalium, 0.21% magnesium dan 0.56% kalsium. Analisa statistik menunjukkan perbezaan bermakna kandungan nutrien abu sekam diantara varieti. Sebagai bahan yang berpotensi digunakan sebagai sumber baja, jumlah nutrien yang boleh didapati dari penggunaan abu sekam dibincangkan.

ABSTRACT

Analysis performed on more than 60 samples of 10 different paddy varieties showed 21.33% of the rough rice comprised rice husk, while 13% of the husk constituted rice husk ash. The nutrient content of rice husk ash was 80.26% silica, 0.38% phosphorus, 1.28% potassium, 0.21% magnesium and 0.56% calcium. Statistically, nutrient composition is significantly influenced by varietal differences. As a potential material for fertilizer use, the estimates of total nutrient supplementation available from rice husk ash per annum are discussed.

INTRODUCTION

Rice is an agricultural crop that continues to be an important source of food and nutrition in Malaysia. The total area planted with paddy in Peninsular Malaysia is 454 917 ha, which constitutes 61.3% of total paddy plantings in the country (Ministry of Agriculture 1993). Net average rice production in the peninsula amounted to 1 810 222 mt in 1992 (Ministry of Agriculture 1993).

A major derivative of paddy is the husk or hull, a fibrous, non-digestible product that comprises approximately 20% by weight of the rough rice. For the period 1991/92, this amounted to 362 044 mt. The most common use of this residue has been the production of heat energy by burning. Due to its abrasive character, poor nutritive value, low bulk density and high ash content, only a small proportion of rice husks has been utilized for non-energy related low value applications, such as chicken litter, animal roughage (Velupillai 1987), mulching and bedding materials (Hsu and Loh 1980). In many Asian countries, where the bulk of rice is produced and consumed, a major proportion of the husks is transported to open fields for disposal by burning. This practice is now strongly opposed and even prohibited in some countries under environment protection legislation. In general, rice husks are residue from the rice processing industry that costs money to dispose of in a manner that does not harm the environ-

ment. One method of turning this liability into an asset is to generate energy from rice husks in a variety of ways. A consequence from these methods is the production of rice husk ash, which is believed to contain various nutrients that enable it to serve as a source of fertilizer.

Rice husk production as a result of milling processes is estimated at 300 000 mt annually. Hence, burning is estimated to produce more than 63 000 mt of ash a year. Based on an estimated content of 1% phosphorus and 1.5% potassium in rice husk ash (Houston 1972), the total phosphorus (P) and potassium (K) which could be obtained exceed 600 mt and 1000 mt p.a. respectively, satisfying the fertilizer requirements of between 20 000 and 48 000 ha of paddy plantings at rates of 30 and 20 kg of P and K per ha respectively.

In the Malaysian context, it would be useful to evaluate the nutritive value of rice husk ash in an effort to create an attractive alternative for the rice processing industry, which could provide a new income source for a rice mill as well as eliminating or greatly reducing space for agricultural wastes. The objectives of this study are to determine the content of various nutrients in rice husk ash and to investigate the influence of rice variety on nutrient content.

MATERIALS AND METHODS

Methodology

Samples, 500 g per variety, of the following varieties: MR 1, MR 27, MR 70, MR 77, MR 82, MR 85, MR 89 and Imp. Mashuri, were obtained from the MARDI Unit, Besut, Trengganu. All samples were selected from uniformly fertilized plants (80 N: 30 P_2O_5: 20 K_2O kg ha^{-1}) and were dried at 70°C for 48 h. Rice husks were separated from the grain using the Satake milling machine, and were accumulated, weighed and stored for analysis.

PREPARATION OF ASH SOLUTION

The method employed was based on Poon (1978) and the Malaysian Standard (SIRIM 1980); 1-g samples of rice husk were heated in a ceramic crucible in a muffle furnace with temperatures increased to 300°C for 1 h and then to 500°C for 10-12 h until the rice husk was transformed into white ash. After cooling, the ash was weighed.

The ash was then moisturized with a few drops of distilled water, mixed with 2 ml concentrated hydrochloric acid, and then dried with periodic heating on a hot plate in a fume chamber at 100-150°C before being mixed with 5 ml nitric acid (20%) and digested on a water bath for 1 h. The mixture was then filtered (using size 2 filter paper) into a volumetric flask. The crucible bowl was washed repeatedly, and the filtrate kept for analysis.

Determination of Nutrient Content in Ash

Silica (Si) was determined gravimetrically using the HCl dehydration method (Willard and Cake 1920; Yoshida 1972). Phosphorus (P) was determined calorimetrically using the vanadomolybdate yellow colour method (Koenig and Johnson 1942) while potassium (K) was determined by flame photometry (Mitchel 1964). Calcium (Ca) and magnesium (Mg) were determined by atomic absorption spectrophotometry method (Wacker *et al.* 1964).

RESULTS AND DISCUSSION

Rice Husk Content in Relation to Varietal Differences

Analysis indicated that percentage of rice husk is significantly influenced by varietal difference at 0.1% level (Table 1). The percentage of rice husk ranged from 23.6% in MR 70 to 20.1% in MR 1. This indicates that different rice varieties produce varying amounts of rice husk.

TABLE 1
Rice husk and rice husk ash content (%) in different varieties

Variety	Rice husk (%)	Rice husk ash (%)
MR 1	20.07^{e}	11.83^{de}
MR 10	21.58^{bc}	12.83^{bcde}
MR 27	21.63^{bc}	14.00^{ab}
MR 70	23.60^{a}	13.83^{ab}
MR 73	20.97^{cd}	14.33^{a}
MR 77	21.15^{cd}	14.00^{ab}
MR 82	20.33^{de}	13.33^{abc}
MR 85	20.27^{de}	12.67^{bcde}
MR 89	21.35^{c}	11.67^{e}
Imp. Mashuri	22.33^{b}	12.00^{cde}
Mean	21.33	13.05

*Values within columns with the same letter are not significantly different at $p < 0.01$ (DMRT)

Total Ash Produced by Burning of Rice Husk

The percentage of ash produced by different varieties was highly significant at 0.1% level, and ranged from 11.7% in MR 89 to 14.3% in MR 73 (Table 1). This compares with 13.2-29.0% reported by Houston (1972).

Relationship between Variety and Nutrient Content in Rice Husk Ash

For all nutrients, there was a significant difference in the percentage composition among varieties (Table 2). The range in these values was compared with values of Houston (1972) for American varieties (Table 3).

In all varieties, percentage of Si was highest. Rice plants are known as accumulators of Si and can contain up to 10% (DW) in husks. Si impregnates the walls of epidermal and vascular tissues (Kitagishi and Yamane 1981), strengthens plant tissues, reduces water loss and retards fungal infection (Tinker 1981). Values of Si are lower for Malaysian varieties, while American varieties show a greater range for P, K, Mg and Ca.

CONCLUSION

Rice husk, which constitutes 21.33% of paddy weight, becomes a waste material of the milling process. Burning of rice husk produces 13% ash, which contains various nutrient elements.

On average, nutrient composition of rice husk ash was 80.26% Si, 0.38% P, 1.28% K, 0.21% Mg and 0.56% Ca. Statistically, differences in the percentages

TABLE 2
Nutrient content of rice husk ash in different rice varieties

Variety	Nutrient (%)				
	Si	P	K	Mg	Ca
MR 1	81.47^{abcd}	0.43^{abc}	1.33^{cd}	0.30^{a}	0.71^{a}
MR 10	74.70^{bcd}	0.36^{bcde}	1.39^{bc}	0.17^{bc}	0.57^{abcd}
MR 27	85.67^{a}	0.36^{bcde}	1.26^{de}	0.12^{c}	0.56^{bcd}
MR 70	84.72^{ab}	0.41^{abcd}	1.50^{a}	0.17^{bc}	0.35^{e}
MR 73	82.35^{abc}	0.34^{cde}	0.88^{f}	0.20^{b}	0.50^{cd}
MR 77	88.52^{a}	0.32^{e}	1.03^{e}	0.21^{b}	0.49^{cd}
MR 82	77.85^{abcd}	0.33^{de}	1.15^{e}	0.20^{b}	0.50^{cd}
MR 85	71.43^{d}	0.38^{abcd}	1.34^{bcd}	0.22^{b}	0.67^{ab}
MR 89	82.02^{abc}	0.46^{a}	1.42^{abc}	0.29^{a}	0.62^{abc}
I. Mash	73.82^{cd}	0.44^{ab}	1.46^{ab}	0.21^{b}	0.60^{abc}

*Values within columns with the same letter are not significantly different at $p < 0.01$ (DMRT).

TABLE 3
Range of percentage composition of nutrients

Nutrient	Malaysian varieties	American varieties[1]
Si	71.43–88.52	91.1–97.0
P	0.32–0.46	0.1–1.3
K	0.87–1.50	0.4–2.5
Mg	0.12–0.30	0.1–1.2
Ca	0.35–0.71	0.2–1.4

([1]Source: Houston 1972)

of these nutrients were highly significant among the different varieties.

These findings suggest rice husk ash is a potential supplementary fertilizer source, convenient for paddy cultivation. From these results, it is estimated that 35 644 mt silica, 169 mt phosphorus, 568 mt potassium, 93 mt magnesium and 248 mt calcium are available annually from rice husk ash. The use of rice husk ash as a fertilizer would also alleviate the problem of its disposal. However, research on formulating cost-effective methods of producing rice husk ash, without aggravating air pollution, is needed.

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Comparative Efficacy of Three Commercial Vitamin and Trace Mineral Premixes for Rearing Broiler Chickens at Starter and Finisher Phases

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Keywords: vitamins, trace minerals, premixes, broiler chickens, diets

ABSTRAK

Enam kumpulan 45 anak ayam panggang Hubbard belum berjantina yang berusia satu hari telah dibandingkan secara rambang mengikut kesamaan pada fasa permulaan (0-5 minggu) kepada tiga diet isoklorik dan diet iso nitrogenus yang dilindungi pada paras yang dicadangkan kepada para pengeluar dengan tiga vitamin komersil dan pracampuran mineral surih yang diperolehi dipasaran tempatan dan dilabel sebagai pracampuran A, U dan Z. Pada fasa penamat (6-9 minggu) anak-anak ayam beralih tempat dibahagi kepada tiga kumpulan rawatan penamat dengan masing-masing dua replika tiap-tiap satu. Satu kumpulan rawatan, berterusan menerima pracampuran yang sama seperti semasa fasa permulaan, sementara dua kumpulan yang lain bersama pracampuran yang selebihnya yang tidak diberi pada fasa permulaan untuk kumpulan rawatan tertentu. Jenis pracampuran yang diberi makan pada fasa permulaan menampilkan perbezaan ($P > .0.05$) dalam pengambilan protein, tambahan berat min dan tambahan berat per unit protein yang diambil oleh burung. Pengambilan protein dan tambahn berat bagi pengambilan per unit protein tidak ketara perbezaannya ($P < 0.05$) pada fasa penamat. Urik serum dan panas kreatinin serum menunjukkan bahawa burung yang diberi makan pracampuran Z pada fasa permulaan menggunakan protein pengimbang dengan lebih berkesan berbanding yang diberi makan dua pracampuran yang lain. Nilai-nilai perubah di perolehi untuk metabolit serum pada fasa penamat. Kesimpulanya, pracampuran yang untuk para pemanggang yang terdapat di Nigeria berbeza dari segi kandungan dan kesan pemakanan protein ayam pemanggang. Adalah dicadangkan bahawa sekiranya kombinasi pracampuran hendak digunakan dalam menternak ayam pemanggang, perhatian perlu ditekankan untuk memberi makanan pracampur yang berkualiti pada fasa permulaan.

ABSTRACT

Six groups of 45 unsexed day-old Hubbard broiler chicks were randomly assigned in duplicate at the starter phase (0-5 weeks) to three isocaloric and iso-nitrogenous diets which were fortified at manufacturers' recommended levels with three commercial vitamin and trace mineral premixes purchased locally and labelled as premix A, U and Z. At the finisher phase (6 -9 weeks) the birds were shuffled and subdivided into three finisher treatment groups of two replicates each. One treatment group continued to receive the same premix as during the starter phase, while the other two groups were assigned the remaining premixes not given at the starter phase for that particular treatment group. Thus nine premix combinations were used at the finisher phase. The premix type fed at the starter phase led to differences ($P > 0.05$) in the protein intake, mean weight gain and weight gain per unit protein intake of the birds. Protein intake and weight gain per unit protein intake were not significantly different ($P < 0.05$) at the finisher phase. The serum uric and serum creatinine levels indicate that birds fed premix Z at the starter phase utilized the dietary protein more efficiently than those fed the other two premixes. Variable values were obtained for the serum metabolites at the finisher phase. It was concluded that available premixes for broilers in Nigeria vary in their content and their effect of protein nutriture of broiler chickens. It is recommended that if combinations of premixes are to be used in rearing broilers, care should be taken to feed a proven premix of good quality at the starter phase.

INTRODUCTION

Optimal use of protein is essential in any practical feeding system. The extent of protein utilization in the diet of broilers is an important factor in determining the rate of growth.

It is well recognized that the nutritive value of the protein content of a diet is affected by the presence and availability of vitamins and minerals that accompany the protein in a diet. Results of an earlier study (Oduguwa and Ogunmodede 1995) indicated that locally available premixes have differing capabilities in supporting the growth of broiler chickens when used as a single premix at two physiological growth stages. It was further noted that the effect of the premix profiles gradually diminished as the birds matured.

The availability of premixes tends to be inconsistent due to the unpredictable economic situation in Nigeria. This necessitates the consideration of employing a combination of premixes for rearing broilers. The present study seeks to explore the complemental effects of using premixes either from a single source throughout or from different sources at the starter and finisher phases.

MATERIALS AND METHODS

Experimental Birds and their Diets

Six groups of 45 unsexed day-old Hubbard broiler chickens were each randomly assigned in duplicate at the starter phase (0-5 weeks) to three isocaloric and isonitrogenous diets. These diets were fortified at manufacturers' recommended levels with three commercial vitamin and trace mineral premixes purchased locally and labelled as premix A, U and Z. At the finisher phase (6-9 weeks) the birds were shuffled and subdivided into three finisher treatment groups of two replicates each. One treatment group continued to receive the same premix as during the starter phase, while the other two groups were assigned the remaining premixes not given at the starter phase for that particular treatment group. (Note that the manufacturer of premix A provided different premixes for starter and finisher stages while the manufacturers of premixes U and Z had only one type of premix for both starter and finisher stages) (Table 1).

The composition of the experimental diets at the starter and finisher phases is shown in Table 2. Feed and water were provided freely. Weekly feed intake and growth rate of the birds were recorded; other parameters were calculated from these records. All birds in each replicate were weighed together and the average weight calculated.

Housing

The experimental birds were reared in tiered brooder cages for the first four weeks of their life before being transferred to a deep litter house. Each compartment in the brooder cages was heated by a 100-watt tungsten filament bulb (white light). The compartments were covered with perforated papers to prevent excessive ventilation and cold. The compartments of the brooder cages (housing 45 birds each) provided adequate drinking and feeding space.

After four weeks, birds were moved into a deep litter house with a short side wall about 1 m high topped with wire mesh, providing uniform-sized pens of about 3.1 m × 1.12 m (for 15 birds). Each pen was equipped with a fountain drinker (3-l capacity) and a rectangular wooden trough feeder. No additional heat or light was provided in the pens after the birds were moved. The ambient temperature of the house during the experiment was 26-32°C.

Nitrogen Retention

Nitrogen retention was determined at 5 and 9 weeks in specially designed metabolic cages equipped with separate watering

TABLE 1
Blood metabolites of the experimental birds at 5 and 9 weeks of age

Parameters	Phase	Premix A			Premix U			Premix Z			S.E.X.
	0-5 weeks (starter)										
Serum total protein (g/dl)		7.10			6.51			5.46			0.55
Serum albumin (g/dl)		6.63[a]			5.64[a]			2.44[b]			0.26
Plasma total protein (g/dl)		7.81[a]			6.49[a]			6.59[b]			0.10
Plasma albumin (g/dl)		6.63			6.21			6.32			0.21
Serum uric acid (mg/dl)		2.08[a]			1.91[b]			1.70[c]			0.02
Serum creatine (mg/dl)		2.42[a]			1.70[b]			1.43[b]			0.07
	6-9 weeks (finisher)	A	U	Z	A	U	Z	A	U	Z	
Serum total protein (g/dl)		6.08[b]	6.00[b]	6.75[a]	5.92[b]	5.71[b]	5.74[b]	5.03[c]	5.38[bc]	4.80[c]	0.72
Serum albumin (g/dl)		4.05[b]	3.99[b]c	4.50[a]	3.94[b]c	3.86[c]	3.86[bc]	3.25[d]	3.95[bc]	3.20[d]	0.06
Plasma total protein (g/dl)		6.82[a]	6.04[a]b	6.69[a]	6.18[a]b	5.83[bc]	5.80[bc]	5.52[bc]	5.34[c]	5.21[c]	0.26
Plasma albumin (g/dl)		5.00[a]	4.04[bc]	5.33[a]	4.92[a]	4.18[b]	4.06[bc]	3.86[bc]	3.84[bc]	3.68[c]	0.13
Serum uric acid (g/dl)		1.43[d]	1.45[a]	1.75[b]	1.25[c]	1.39[de]	1.49[cd]	2.01[a]	1.62[bc]	1.62[bc]	0.04
Serum creatine (mg/dl)		2.45[c]	2.12[c]	2.80[b]	1.88[f]	2.08[e]	2.24[d]	3.02[a]	2.44[c]	2.43[c]	0.03

abcd Values with different superscripts on the same row were significantly different ($P < 0.05$)

troughs and feeders. For each metabolic trial, two birds were randomly selected from each replicate and housed together in a compartment.

A 4-day acclimatization period was allowed prior to a 3-day collection period. The weight of feed given to each group was recorded and the feeds were maintained at low levels in the trough in order to avoid spillage. During the collection of excreta, 1% boric acid solution was sprayed on the droppings regularly to prevent the escape of nitrogen. The total droppings voided from each replicate were then thoroughly mixed together in separate basins. Weighed representative samples for each were then taken in well labelled aluminum foil packets. These labelled packets containing

TABLE 2
Composition of experimental diets

Ingredients g/kg of diet	Starter Rations			Finisher Rations		
	Premix A	Premix U	Premix Z	Premix A	Premix U	Premix Z
Yellow corn	560.0	560.0	560.0	558.0	558.0	558.0
Groundnut meal	214.0	214.0	214.0	186.9	186.9	186.9
Spent grain	68.0	68.0	68.0	120.1	120.1	120.1
Menhaden fish meal	40.0	40.0	40.0	35.0	35.0	35.0
Blood meal	45.0	45.0	45.0	30.0	30.0	30.0
Palm oil	20.0	20.0	20.0	15.0	15.0	15.0
Bone meal	35.0	35.0	35.0	35.0	35.0	35.0
Oyster shell	10.0	14.0	12.5	10.0	14.0	12.5
Salt	3.0	3.0	3.0	5.0	5.0	5.0
*Vitamin/mineral premix	5.0 1000	1.0 1000	2.5 1000	5.0 1000	1.0 1000	2.5 1000
Determined Analysis						
Crude protein (g/kg diet)	221.8	231.2	228.6	199.6	203.5	202.4
Crude fibre (g/kg diet)	47.7	49.4	48.7	51.2	50.0	48.8
Ether extract (g/kg diet)	54.3	57.6	59.7	54.7	51.6	53.1
Ash (g/kg diet)	75.2	66.8	90.3	69.2	71.8	72.3
Calculated Analysis						
Energy (ME) Kcal/kg	3024.5	3024.5	3024.5	2998.0	2998.0	2998.0

wet droppings were dried in the oven at 65° to determine the moisture content, and hence the dry matter, for each. Collection was on a daily basis so the above procedure was repeated for the other two collection days. Dry droppings from the same replicates were then thoroughly pooled and ground. The dried, pooled and ground samples were stored in well labelled and covered glass bottles for laboratory analyses. The nitrogen retention was calculated by the formula

$$\text{Percentage Retention} = \frac{\text{nitrogen N2 in feed} - \text{N2 in droppings}}{\text{N2 in feed}}$$

Blood Metabolites

Blood samples were collected and analysed for serum total protein, serum albumin, serum acid creatinine, plasma total protein and plasma albumin when the birds were five and nine weeks old. Six birds were randomly selected from each replicate and two sets of blood samples were collected from the birds in each replicate. Samples of blood (4 ml) were taken by a careful puncture of the jugular vein. Samples for serum analyses were decanted after centrifugation while EDTA was added to the blood samples and used for plasma analysis, which was carried out within four hours of collection. The decanted serum samples were stored in a freezer for subsequent analysis.

Serum and plasma total protein were determined by the biuret method of Reinhold (1953) while the determination of serum and plasma albumin was by bromocresol green binding reagent method of Doumas and Briggs (1972). The phosphotungstate method of Caraway

(1963) was used to determine uric acid. Serum creatinine determination was by folin Wu filtrate method. The data obtained were subjected to analyses of variance and significant differences between means were evaluated using Duncan's multiple range test (Gomez and Gomez 1984). All statements of statistical significance were based on $P < 0.05$.

RESULTS AND DISCUSSION

The premixes varied in the number and quantity of the vitamins and trace minerals present (Table 3). The differences observed between dietary treatments were probably due to these differences since the premixes were the only variable.

At the starter phase, birds on the diet fortified with premix Z gained more weight than those fed on diets containing the other two premixes (Table 4). The weight gain is an expression of the effect of the intake and utilization of protein. Table 3 shows that premix Z contains adequate levels of all the vitamins and trace minerals listed by NRC (1984). Premix A lacks micronutrients such as vitamin B12, zinc, biotin, folate, vitamin K, pantothenate, cobalt and thiamin. Vitamin B12, zinc and folate (Akesson *et al.* 1982) are all known to play important roles in the metabolism of protein, and their absence in premix A would have impaired protein utilization by the birds fed this premix. This is evident from the fact that the birds fed premix A diet ate as much as those on premix Z diet but gained less at the starter phase. Premix U probably could not stimulate enough protein intake to allow the broilers to fully express their genetic potential. The utilization of protein

TABLE 3
Micronutrients in various premixes (per kilogram of feed)

Premix	A	A	U	Z	*NRC requirement (per kg of feed)
	Starter		Finisher		
Vitamin A(IU)	18000	15000	8000	12500	1500
Vitamin D(IU)	2500	2500	1500	2500	200
Vitamin E(IU)	14	11	3	40	10
Vitamin B_2 (mg)	12	10	2.5	6	3.6
Vitamin B_3(mg)	44	40	8	35	27
Vitamin B_6(mg)	28	20	0.3	3.5	3.0
Choline chloride (mg)	480	400	–	300	1300
Manganese (mg)	120	120	10	100	60
Iron (mg)	70	70	5	50	80
Copper (mg)	10	10	0.2	2.0	8.0
Iodine (mg)	2.2	2.2	0.15	1.55	0.35
Selenium (mg)	0.2	0.2	0.01	0.10	0.15
Vitamin K_a (mg)	–	–	3	2.5	0.50
Calcium pantothenate (mg)	–	–	3	10	10
Vitamin B_{12} (mg)	–	–	0.008	0.025	0.009
Zinc (mg)	–	–	4.5	45	40
Cobalt (mg)	–	–	0.02	0.225	–
Vitamin B_1 (mg)	–	–	–	2.0	1.80
Biotin (mg)	–	–	–	0.05	0.15
Folio acid (mg)	–	–	–	1.00	0.55

* NRC 1984

TABLE 4
Mean protein intake growth rate and nitrogen retention of experimental birds at starter and finisher phases

Parameter	Phase	Premix A			Premix U			Premix Z			S.E.X.
	0-5 weeks (starter)										
Protein intake (g/day)		10.06^{a}			8.33^{b}			10.71^{a}			0.53
Weight gain (g/day)		14.04^{b}			10.58^{c}			16.81^{a}			1.71
Weight gain/ protein intake		1.40^{a}			1.27^{b}			1.58^{a}			0.16
Nitrogen retention (%)		50.27			49.43			42.96			8.25
	6-9 weeks (finisher)	A	U	Z	A	U	Z	A	U	Z	
Protein intake (g/day)		20.26	19.15	20.05	18.52	20.00	21.95	18.67	19.50	21.58	1.00
Weight gain (g/day)		26.01^{ab}	26.21^{ab}	27.79^{ab}	25.30^{b}	23.31^{c}	26.04^{ab}	25.70^{ab}	25.95^{ab}	30.11^{a}	1.78
Weight gain/ protein intake		1.28	1.39	1.39	1.37	1.17	1.19	1.38	1.33	1.40	0.1
Nitrogen retention (%)		47.53^{b}	48.93^{b}	57.12^{ab}	60.54^{ab}	71.87^{ab}	64.40^{ab}	65.83^{ab}	59.42^{ab}	70.07^{a}	4.70

abc Values with different superscripts in the same row were significantly different ($P < 0.05$)

by birds fed on the diet containing this premix was the lowest among the three treatment groups as indicated by the values for weight gain and the weight gain per unit protein intake.

There was no difference ($P > 0.05$) between treatments in protein intake at the finisher phase (Table 4). The complemental effects of a combination of the premix profiles was probably an important factor in stabilizing the protein intake at this phase. Endogenous secretion of vitamins (thiamin, niacin and pyridoxine) which are known to affect protein appetite in maturing birds should also be recognized as an important factor in stabilizing protein consumption. Birds fed premix U diet at both phases weighed less than those fed diets containing premix A or Z in both phases. There was no difference in nitrogen retention value between treatments at the starter phase. The high nitrogen retention values of birds fed premix U diet in both phases was not reflected in their live weight.

Birds fed premix A diet at the starter phase had consistently high protein levels in the plasma and serum fractions. This is consistent with findings of an earlier study (Oduguwa and Ogunmodede 1995). High levels of transaminating enzymes causing high dietary vitamin B6 (Chen and Marlatt 1975; Saroka and Combs 1986) was the reason adduced for the high protein level in the serum of birds fed on the diet with premix A. Birds given premix A diet at the starter phase and finished with premix Z diet had the highest values for serum total proteins and albumin fractions. In fact, treatment birds started with premix A diet and finished with any of the premix types had relatively high levels of both serum and plasma total protein and albumin fraction than treatment birds that were started with other premix types.

Uric acid metabolism is influenced by the amount of protein and amino acids in the diet. Serum uric acid (Morgensten *et al.* 1960) and creatinine (Eggum 1970) can be used as an indirect measure of protein adequacy. Treatment birds were fed iso-nitrogenous and isocaloric diets, but the group that received premix Z gained more weight at the starter phase and also had the lowest levels of uric acid in their serum. This indicates that more nitrogen/protein was incorporated into the body protein and less was excreted as uric acid. The same deduction could be made on the low creatinine levels obtained for the group of birds. Those fed with premix U diet at the starter phase and finished with premix A diet had consis tently low levels of serum uric acid and serum creatinine but the birds did not have the highest mean weight gains. The interaction of the various micronutrients in the combination of premix profiles in the bird at this stage (finisher phase) might have affected the metabolite levels which did not show in the weight changes.

The results of the study indicated that the available premixes for broilers in Nigeria vary widely in their content and thus their effects on the protein nutrition of broiler chickens, and that younger birds tend to be more sensitive to the vitamin and trace mineral profile fed to them. Therefore if combinations of premixes are to be used in rearing broilers, care should be taken to feed a proven premix of good quality at the starter phase.

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Effect of Ripe Plantain Peel (*Musa* cv) on Growth and Carcass Performance of Growing Rabbits

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ABSTRAK

*Percubaan memakan telah dilakukan dengan mengguna 48 ekor arnab penyapih New Zealand White × Flemish Giant bagi menentukan nilai penggantian jagung kepada kulit pisang masak (*Musa *cv) dalam pemakanan arnab penyapih. Kulit pisang masak telah diguna untuk menggantikan jagung pada 0, 33, 66 dan 100% dalam amalan pemakanan penyapih. Hitung panjang pertambahan harian dan makanan/berat menurun ($P<0.05$) pada 100% paras penggantian. Bahan kering, protein mentah dan koefisian ketercernaan tenaga adalah bererti ($P<0.05$) menurun oleh kandungan kulit pisang masak di dalam pemakanan. Bagaimanapun, kandungan kulit pisang masak tidak mempunyai kesan bererti terhadap hitung panjang pengambilan makanan dan ketercernaan serat mentah. Peratus campuran, berat bahagian perut dan berat dada juga menurun ($0<0.05$) oleh 100% paras penggantian kulit pisang masak, manakala kandungan kulit pisang masak dalam permakanan meningkatkan ($P<0.05$) berat visera.*

ABSTRACT

A feeding trial was conducted using 48 New Zealand White x Flemish Giant weaner rabbits to determine the replacement value of ripe plantain peel for maize in weaner rabbit diets. Ripe plantain peel was used to replace maize at 0, 33, 66 and 100% in a practical weaner diet. Average daily gain and feed/gain were depressed ($P<0.05$) at the 100% replacement level. Dry matter, crude protein and energy digestibility coefficient were significantly ($P<0.05$) depressed by inclusion of ripe plantain peel in the diet. However, the inclusion of ripe plantain peel had no significant effect on average feed intake and crude fibre digestibility. Dressing percentage, lumbar region weight and breast weight were also depressed ($P<0.05$) by the 100% replacement level of ripe plantain peel, while inclusion of ripe plantain peel in the diet increased ($P<0.05$) the viscera weight.

INTRODUCTION

Over 60% of the world's plantain is produced and consumed in West and Central Africa (IITA 1987). Plantain (*Musa* cvs) is a popular Nigerian staple food from which various dishes are prepared and peel is generated in large quantities. In most cases its disposal constitutes a problem. Olayide *et al.* (1972) estimated the annual production of plantain products in Nigeria at almost 1.5 million metric tons.

Dairo *et al.* (1987) reported the possibility of using plantain peel as a source of energy. The proximate analysis of peel compares favourably with maize except in crude fibre and ether extract (Ketiku 1973). Peel also contains higher levels of minerals such as calcium, iron and phosphorus. Dairo *et al.* (1987) have shown that plantain peel can constitute as much as 5% of a layer diet.

Conscious of the need to identify and evaluate new cheap sources of feed ingredients, the potential of ripe plantain peel was investigated as a source of energy in

TABLE 1
Chemical composition and energy value of dried ripe plantain peel (%)

Crude protein	9.83
Crude fibre	5.63
Ether extract	14.23
Ash	13.16
Calcium	0.96
Phosphorus	0.32
Energy (ME) (MJ)	13.96

rabbit rations. This study was designed to evaluate the replacement value of ripe plantain peel for maize in rations for growing rabbits.

MATERIALS AND METHODS

Diets

Plantain peel collected from a plantain chip factory in Lagos 24 hours after peeling was sun-dried for 5 days and ground in a hammer mill. Samples of dried plantain peel (DPP) meal were analysed for proximate chemical composition (AOAC 1990). Based on the results of the chemical analysis (Table 1), 4 experimental diets were formulated with plantain peel meal replacing maize at 0, 33, 66 and 100%, respectively. Table 2 shows the composition of the diets.

Animals

Forty-eight 6-week-old weaner rabbits (New Zealand White × Flemish Giant), with a mean weight of 0.58 ± 0.05 kg, were randomly assigned to the 4 dietary treatments on the basis of initial weight and sex, with 12 rabbits per treatment. Each treatment was replicated 4 times with each replicate group of 3 rabbits housed in a hutch of 180 × 45 cm. Feed and water were freely available. Rabbit weight and feed consumption were recorded weekly. Proximate analysis of the treatment diets was determined following methods of AOAC (1990). Energy was determined with a ballistic bomb calorimeter in which benzoic acid was used as standard. Mineral analyses were made by the methods of

TABLE 2
Formulation and chemical composition of dried plantain peel (DPP)

	% Replacement level of DPP in diets			
	0	33	66	100
Formulation				
Maize	300	200	100	–
Dried plantain peel	–	100	200	300
Full-fat soyabean	150	150	150	150
Palm kernel cake	100	100	100	100
Dried brewer's grain	430	430	430	430
Bone meal	12.5	12.5	12.5	12.5
Oyster shell	2.5	2.5	2.5	2.5
Salt	2.5	2.5	2.5	2.5
*Premix	2.5	2.5	2.5	2.5
Chemical analysis (fresh weight basis)				
Dry matter	906.0	865.0	900.0	898.0
Crude protein	178.6	180.0	180.6	181.4
Ether extract	79.6	84.6	99.4	110.2
Crude fibre	123.6	130.5	129.5	139.5
Gross energy (MJ/kg^{-1})	16.6	16.6	16.3	16.2

TABLE 3
Digestibility coefficients of ripe plantain peel-supplemented diets (%)

	Plantain peel replacement level (%)				Significance	SEM
	0	33	66	100		
Dry matter	89.12	86.51	84.35	79.48	*	0.11
Crude protein	80.10	79.16	78.79	76.10	*	0.06
Crude fibre	59.46	58.86	59.12	58.14		0.04
Energy	81.53	80.82	79.67	77.11	*	0.02

Grueling (1966). The animals were fed for 7 days before collection of faecal material for a digestibility trial. Digestibility coefficients for dry matter, crude protein, crude fibre and energy were determined for each diet (Table 3). The experiment lasted for 8 weeks. At the end of the trial, 4 animals per treatment were weighed, slaughtered and their viscera removed. The weight of liver, heart, kidneys and cut parts was determined.

Statistical Analysis

Data collected were subjected to analysis of variance as outlined by Snedecor and Cochran (1978). When analyses of variance indicated a significance for treatment effects, specific differences between means were detected by Duncan multiple range test (Duncan 1955).

RESULTS AND DICUSSION

The chemical composition of dried plantain peel compares favourably with maize except in crude fibre and ether extract (Ketiku 1973). Peel is higher in Ca and P.

Performance data are given in Table 4. Feed consumption did not differ significantly ($P > 0.05$) between dietary treatments. Feed consumption increased slightly with level of ripe DPP in the diet. This slight increase could have resulted from the higher crude fibre, lower caloric density (Beynen 1988) and probably better palatability of the ripe DPP as a result of its simple sugars (Ketiku 1973). Beynen (1988) observed that high caloric density diets result in decreased feed intake by rabbits.

Average daily liveweight gain (ADG) was significantly ($P < 0.05$) lower for rabbits fed the diet containing 100% replace-

TABLE 4
Effect of dried ripe plantain peel on rabbit performance

	Plantain peel (%)				SEM[1]
	0	33	66	100	
Initial liveweight (kg)	0.58	0.59	0.58	0.58	0.01
Final liveweight (kg)	1.39	1.48	1.39	1.20	0.04
Body weight changes (g)	850[a]	890[a]	810[a]	690[b]	10.40
Daily weight gain (g)	15.18[a]	15.89[a]	14.46[a]	12.32[b]	0.11
Feed/gain	4.08b	3.95b	4.37b	5.31a	0.11
Feed cost (N/kg feed)	9.73	9.13	8.53	7.93	–
Feed cost savings (%)	–	6.17	12.33	18.50	–

[1] Standard error of the means

a,b,: Means on the same row not followed by the same letter are significantly different ($P < 0.05$)

TABLE 5
Carcass yield (% liveweight) of rabbits fed different levels of ripe plantain peel

	Replacement level of plantain peel (%)				SEM
	0	33	66	100	
Liveweight (g)	1100	1050	1200	1000	0.94
Dressing (%)	68.18[a]	61.90[ba]	63.33[a]	55.00[b]	0.05
Viscera weight	22.73[b]	30.00[a]	29.17[a]	35.00[a]	0.85
Lumbar region weight	9.09[a]	8.00[a]	7.50[a]	5.00[b]	0.10
Head	9.09	10.00	8.33	10.00	0.77
Hind limbs	16.36[a]	13.00[b]	12.10[b]	12.00[b]	0.65
Fore limbs	9.09[a]	8.00[a]	6.67[b]	8.00[a]	0.11
Breast	4.55[a]	4.00[a]	3.92[a]	3.00[b]	0.22
Liver	3.28	3.1	3.01	2.79	0.03
Kidney	0.80	0.75	0.73	0.70	0.02
Heart	1.09	0.78	0.92	0.76	0.04

a,b: Means on the same row not followed by the same letter are significantly different ($P < 0.05$)

ment level of ripe DPP (Table 5). Differences between the 0, 33 and 66% replacement levels of DPP were, however, non-significant ($P > 0.05$). The ADG values were generally lower than normal values, but conformed with the general trend in developing countries (Cheeke 1986; Aduku *et al.* 1988; Alawa *et al.* 1989; Balogun and Etukude 1991).

The best feed conversion efficiency (FCE) was recorded with the 33% replacement level of ripe DPP. The 100% ripe DPP significantly ($P < 0.05$) reduced the efficiency of feed conversion despite the consumption of more food by rabbits on this diet. This suggests that the diet was less adequate in nutrient content and quality. This is in agreement with the ADG results.

Rabbits fed on the 0% replacement level of ripe DPP had higher ($P < 0.05$) DM, crude protein and energy digestibility coefficients than rabbits fed on the diets containing ripe DPP. Digestibility coefficient decreased with increased level of ripe DPP in the diet. Digestibility of protein has been shown to be adversely affected by the presence of crude fibre (Sauer *et al.* 1980) and tannin (Clandinin and Heard 1968).

Dressing percentage, lumbar region weight and breast weight were significantly reduced by 100% replacement level of ripe DPP in the diet, while DPP in the diet increases ($P < 0.05$) the viscera weight. The average dressing percentage was similar to values reported by Rao *et al.* (1977) for rabbits at 12 and 16 weeks of age. No significant difference ($P < 0.05$) existed in the weights of the various organs.

Feed cost analysis of the diets showed feed cost savings of 6.17, 12.33 and 18.50%, respectively, for 33, 66 and 100% replacement levels.

CONCLUSION

The results of this study suggest that ripe DPP can replace up to 66% of maize (20% ripe DPP inclusion) in diets for weaner rabbits without significantly affecting their performance.

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