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The Distribution of the Heavy Metals (Cu, Pb and Zn) in the Soft and Hard Tissues of the Green-Lipped Mussel *Perna viridis* (Linnaeus) Collected from Pasir Panjang, Peninsular Malaysia

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Keywords: *Perna viridis*, heavy metal distributions, soft tissues, shell, Malaysia

ABSTRACT

The distributions of Cu, Pb, and Zn in the different soft Pasir Panjang, Peninsular tissues, shell sections and layers of the green-lipped mussel *Perna viridis* from an area with unpolluted water in Malaysia, were studied. The soft tissues analysed were the byssus, mantle and gills, posterior adductor muscle, retractor byssal muscle, foot, crystalline style, gonad and the remaining visceral mass. The level of Cu in the crystalline style was significantly ($P < 0.05$) elevated when compared to the other soft tissues while elevated levels of Cu, Pb and Zn were found in the byssus. The byssus is therefore a more sensitive material for Cu, Pb and Zn. The heavy metal concentrations in the different sections of the mussel's shell layers were also differed. The level of Pb was significantly ($P < 0.05$) higher in the inner shell layer than in the periostracum layer while Cu and Zn concentrations were significantly ($P < 0.05$) higher in the periostracum layer than in the inner shell layer. Copper, Pb and Zn were evenly distributed within the different sections of the inner shell layer with no significant ($P > 0.05$) difference in the concentrations of these metals in the different sections. The periostracum shell layer was found to be a more sensitive biomonitoring material for Cu and Zn than the inner shell layer.

INTRODUCTION

In a 'Mussel Watch' approach, the determination of accumulated concentrations of heavy metals by using the total soft tissues in the mussels as integrated measures of ambient metal bioavailabilities had been a common practice (Goldberg, 1975, 1980; Goldberg *et al.*, 1978; Phillips, 1980, 1985, 1991; Phillips and Segar, 1986; Phillips and Rainbow, 1993; Rainbow and Phillips, 1993). However, the metal concentrations in the total soft tissues of the organism may not be accurately reflective for certain contaminant concentrations in individual target tissues of the organism. This argument was based on the fact that different tissues accumulate metals at different rates and that the biological half-lives of metals at each type of soft tissue also differ from one another (Lakshmanan and Nambisan, 1989). This is due to the different capacities of the cells in each type of tissue to eliminate the metals bound to the binding sites of the

metallothioneins (Viarengo *et al.*, 1980, 1985). This useful detoxification mechanism is why certain bivalves could survive with elevated levels of heavy metals accumulated in their soft tissues. The metallothioneins and granules could prevent interference by heavy metals of the basal metabolic roles and damage to the cellular structures (Viarengo *et al.*, 1985). This is because metals bound to metallothioneins represent a non-toxic form of the metal itself as shown by several researchers (Roesijadi and Robinson, 1994; Rainbow, 1997). Since each soft organ may play a different role either in its metabolic or physiological function, this may influence the distribution of metals in the different soft tissues of mussels. As a result, the metal regulation and detoxification processes could also be different. Knowledge on metal distributions in the soft tissues may help us to understand the processes involved in the uptake and excretion of metals in the different soft tissues of *P. viridis*.

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The distribution of metals in the hard tissues (periostracum and inner shell layers) of *P. viridis* was also studied. The whole shell of *P. viridis* consists mainly of the inner shell layers (prismatic and nacreous) with very superficial layer of organic periostracum layer that covers the inner shell layers (Kennedy *et al.*, 1969; Bubel, 1976). According to Phillips (1980), the adsorption of metals onto the shell surface caused difficulty in using mussel shell as a biomonitoring material for heavy metals. Several authors had investigated the suitability of mussel shell as a biomonitoring material for heavy metals (Pilkey and Goodell, 1963; Bertine and Goldberg, 1972; Goldberg, 1975, 1980; Imlay, 1982; Koide *et al.*, 1982; Szefer and Szefer, 1985; Dermott and Lum, 1986; Bourgoin, 1987). Foster and Chacko (1995) suggested that shells could accumulate a wide range of metals to varying extents. The accumulation of heavy metals in the shells had prompted some investigators (Carriker *et al.*, 1980, 1982; Koide *et al.*, 1982; Al-Dabbas *et al.*, 1984) to develop mollusks shells as a biomonitoring material for heavy metals. They believed that the chemical composition of the shells might be a good reflection of the environmental levels of heavy metals. Several researchers (Sturesson, 1976, 1978; Carell *et al.*, 1987) also suggested the possible use of shell material as a permanent record of environmental changes in heavy metal contamination.

This study aimed to determine the distribution of Cu, Zn and Pb in the different soft tissues of the green-lipped mussel *P. viridis* from an unpolluted area of Malaysian coastal waters and to identify the potential biomonitoring organ for further studies. Besides that, the levels of heavy metals in the different layers and sections of the hard tissues (shell) of the mussel *P. viridis* were also studied.

MATERIALS AND METHODS

Samples of *P. viridis* were collected from an area of unpolluted water off the west coast of Peninsular Malaysia (Pasir Panjang). All samples were stored in polyethylene bags at -10°C until analysis. Before dissection, the samples were thawed at room temperature on a clean tissue paper with the posterior margins downward to drain away excess water. About 30 individuals of a similar size group (shell length 7-8 cm) were selected for metal analysis. The total soft tissues of *P. viridis* were carefully removed by deshell-

ing the mussels with a stainless steel knife. The soft tissues were then dissected into 8 parts namely byssal threads, mantle and gills, posterior adductor muscle, retractor byssal muscle, foot, crystalline style, gonad and remaining visceral mass. The latter fraction contained about half of the total weight of the mussel. Three parts (mantle and gills, gonad and remaining visceral mass) were analysed by sex. The sex of *P. viridis* was distinguished based on the colour of the gonads (Yap *et al.*, 1979). The different soft tissues were pooled in order to get enough samples for metal analysis. Excess water was pressed on a filter paper. The fresh tissue was then ready for further analysis.

The shells were cleaned under a jet of tap water to remove the algae, barnacles, mud and bryozoa encrusted on them. All samples were rinsed with double distilled water (ddw) and 0.5% HNO₃, and dried for 72 hours at 105°C to a constant weight (Mo and Neilson, 1994). In order to separate the outermost layers (periostracum layers) of the shells, the shells were cooled at room temperature after heating at 105°C. While the shells were cooling, most of the periostracum on the lip part of the shell cracked and fell off (Puente *et al.*, 1996). The different sections of the inner nacreous layer, as shown in Fig. 1, were analysed. These shell sections included the umbo, green-lipped part, ligament, ventral and dorsal shell parts. Pestle and agate were used to crush the hard inner nacreous shell layers into smaller pieces. All samples were weighed with an accuracy of 0.1 mg before acid digestion.

All samples were digested in concentrated HNO₃ (AnalaR grade, BDH 69%). They were placed in a hot-block digester first at low temperature for one hour and then they were fully digested at high temperature (140°C) for at least 3 hours. The digested samples were then diluted to a certain volume with ddw. After filtration, the prepared samples were determined for heavy metals by using an air-acetylene flame atomic absorption spectrophotometer (AAS) Perkin-Elmer Model 4100. The data are presented in either mg/g wet or dry weight basis. To avoid possible contamination, all glassware and equipment used were acid-washed and the accuracy of the analysis was checked against the procedural blanks and standard addition testing procedure. The percentages of recoveries for the heavy metal analyses were

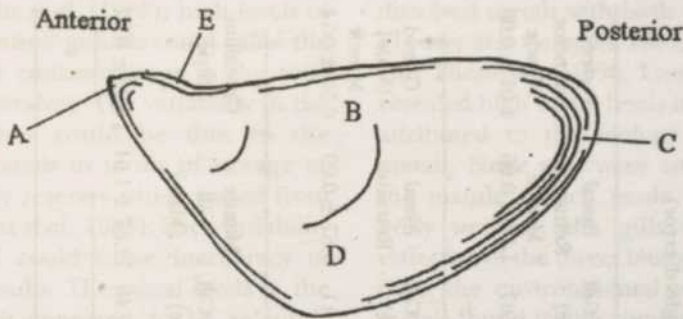


Fig. 1: Different sections of the shell of the mussel *P. viridis* used in analysis (A: the tip and the anterior shell or umbo; B: near dorsal shell part; C: green-lipped part posterior of the shell; D: near ventral shell part; E: ligament). Note: Periostracum is not shown in this drawing because this outer shell layer can only be shown in green color on the outer layer of the shell

96% for Cu, 92.5% for Pb and 92% for Zn. One-way ANOVA Student-Newman-Keuls test (Day and Quinn, 1989) was used to elucidate where differences occurred. All the comparisons were made at the 95% ($P < 0.05$) level of significance.

RESULTS AND DISCUSSION

Different Soft Tissues

The heavy metal concentrations in each soft tissue analysed were shown in Table 1. The distributions of Cu, Pb and Zn were determined from mussel samples collected from the field. 'Why did the metals distribute unevenly in the different soft tissues?' is an interesting question. Some tissues accumulated significantly higher ($p < 0.05$) levels of Cu, Pb and Zn than the other soft tissues. This could be due to each organ playing a different role in metabolism and physiological functions. The results indicated that the regulation and detoxification of metals were also different in each soft tissue.

The crystalline style accumulated significantly ($p < 0.05$) higher levels of Cu when compared to the other soft tissues (Table 1). High levels of Cu in the crystalline style may be due to a metabolic function in the digestive system of *P. viridis*. The crystalline style is the main organ of the digestive alimentary canals (Morton, 1992). It functions in the detoxification of metals and has other enzymatic activities (Phillips and Rainbow, 1993). Selvarani *et al.* (1989) revealed that the crystalline style in *P. viridis* exhibited strong amylase activity which was double that of the digestive diverticula. Since Cu is an essential element, most of the Cu was bound to metallothionein and therefore the Cu level in the crystalline style was high.

Generally, the byssus of *P. viridis* accumulated high levels of Cu, Pb and Zn when compared to the other soft tissues. This indicated that the materials available for the production of the byssal threads contained a lot of metal wastes. Cheung and Wong (1992) also recorded high metal concentrations in the byssus, particularly for Cu and Cd. This result indicated that the byssus could have been used for metal excretion (Pentreath, 1973; Goldberg *et al.*, 1978). The formation of the byssal threads requires the expenditure of a substantial amount of energy and material (Coomb and Keller, 1982). The byssus is excreted from the byssal glands at the foot and is composed of hard tanned protein (Ikuta, 1986). The byssus of *Mytilus* spp. was found to be a good biomonitoring material for Cu (Szefer *et al.*, 1997) and Hg (Szefer *et al.*, 1999).

The metal levels in the foot were lower than in the byssus and other soft tissues (Table 1). The low levels of metals in the foot are expected since this material is probably acting as a channel to transport all the waste materials to the byssal threads (Morton, 1992). Thus, the foot would hardly accumulate any metal. In addition, the foot has a lower volume of surface contact when compared to the gills.

The gonad accumulated considerable metal levels when compared to the other tissues (Table 1). The gonadal tissue is usually related to the condition of the mussel in that the physiological condition of the mussel is reflected through the amount of gonads merged with the mantle (Peddicord, 1977). In this study, the female gonad accumulated higher Pb and Zn concentrations than the other soft tissues.

TABLE 1
Student-Newman-Keuls (SNK) comparisons of Cu, Pb and Zn concentrations (means mg/g wet weight \pm standard error)
in different tissues of the mussel *P. viridis*

Organs	Crystalline Style	Byssus	Gonad (Male)	Gonad (Female)	Remaining Visceral Mass (Female)	Remaining Visceral Mass (Male)	Mantle + Gill (Male)	Foot	Mantle + Gill (Female)	Byssal Retractor Muscle	Posterior Adductor Muscle
Means \pm SE Cu	6.36 \pm 1.13	4.57 \pm 0.54	2.64 \pm 0.08	2.63 \pm 0.19	2.46 \pm 0.07	2.36 \pm 0.14	2.13 \pm 0.34	1.76 \pm 0.05	1.50 \pm 0.07	1.13 \pm 0.06	1.01 \pm 0.05
Organs	Byssus	Remaining Visceral Mass	Gonad (Female)	Remaining Visceral Mass (Female)	Foot (Male)	Posterior Adductor	Crystalline Style Muscle	Mantle + Gill (Male)	Mantle + Gill (Female)	Byssal Retractor	Gonad (Male) Muscle
Means \pm SE Pb	2.70 \pm 0.13	1.38 \pm 0.10	1.10 \pm 0.07	1.07 \pm 0.10	0.93 \pm 0.13	0.85 \pm 0.24	0.79 \pm 0.09	0.71 \pm 0.04	0.69 \pm 0.11	0.67 \pm 0.09	0.59 \pm 0.14
Organs	Remaining Visceral mass (Female)	Gonad (Female)	Byssus	Remaining Visceral Mass (Male)	Posterior Adductor Muscle	Gonad (Male)	Byssal Retractor Muscle	Foot	Mantle + Gill (Female)	Mantle + Gill (Male)	Crystalline Style
Means \pm SE Zn	23.98 \pm 1.17	21.79 \pm 0.83	19.56 \pm 2.11	17.25 \pm 0.75	16.59 \pm 0.60	16.01 \pm 1.28	15.87 \pm 0.32	14.39 \pm 0.76	14.19 \pm 0.46	12.87 \pm 1.01	3.92 \pm 0.32

Note: Means not differing significantly at $P < 0.05$ are indicated by a line under the corresponding values.

According to Wright *et al.* (1985), high levels of heavy metals in bivalves' gonads could cause the variability of metal concentrations in the total soft tissues of the bivalves. The variability in the metal accumulation could be due to the development of gonads in terms of storage or depletion of energy reserves which varied from time to time (Wright *et al.*, 1985). Such variability in levels of metal could cause inaccuracy in ecotoxicological results. The metal levels in the gonad of *P. viridis* appeared to be relatively higher than in the rest of the soft tissues analysed which corroborates the findings of Yang and Thompson's (1996). Yang and Thompson (1996) demonstrated that the distribution of Cu in the different organs of the mussel *P. viridis* was in the order gill > gonad > visceral mass > mantle > muscle whereas for Zn it was visceral mass > gonad > gill > muscle > mantle. The higher metal levels in the female's gonads compared to the male's gonads especially for Zn was perhaps due to gamete production in the female gonads that contained stored reserves with more metals (Bayne *et al.*, 1982). The metal levels in the other soft tissues were relatively similar and were not significantly ($P > 0.05$) different from one another.

Table 1 also shows that the metal concentrations in the remaining visceral mass were relatively higher than those in the other soft tissues. The relatively higher concentrations of Zn and Pb found in the remaining visceral mass may be due to the high rate of uptake and the subsequent loss of these metals in the fecal materials. This remaining visceral tissue also included the hepatopancreas and the kidneys which could contribute to the high metal concentrations. This part also contained a lot of plankton and algae which were kept in this part before further digestion. Therefore, it reflected the main food of mussels obtained through feeder-filtering activities which directly contributed to the metal uptake of the mussels.

Mantle and gills showed relatively low levels of metal concentrations when compared to other soft tissues (Table 1). This may indicate that the mussels from this population had relatively little gonads that merged with the mantle since the gonads accumulated considerable amount of metals. Since mantle and gill are in contact with large volumes of water necessary for feeding and respiration, the metals detected in these organs probably resulted from the interaction of

dissolved metals with both sediment and mussel (Tessier and Campell, 1987). Some studies (Yang and Thompson, 1996; Lares and Orians, 1997) revealed high metal levels in the gills which were attributed to the biology and metabolism of metals. Since gills were analysed together with the mantle (which made up most of the soft body weight), the gills therefore were less reflective of the direct biological uptake of metals from the environmental seawater. As a result, metals found in the combination of mantle and gills were mainly due to the mantle since it made up the bulk the weight.

The distribution of metals in different soft tissues of *P. viridis* could also be due to the different biological half-lives of Cu, Pb and Zn. These were related to the differing capacities of the cells to eliminate the metals bound to metallothioneins (Viarengo *et al.*, 1980, 1985). The fact that the three metals, had been sequestered in different concentrations in different soft tissues, was probably due to the fact that these metals and the metallothioneins associated with them followed different biochemical pathways. Therefore, the determination of metals in the different soft tissues can be used as a method to monitor the detoxified forms of metals which had previously been accumulated in these tissues under field conditions.

Shell

For the shell of *P. viridis*, varying distributions of Cu, Pb and Zn were also found in different sections of the inner shell layer but the differences were not significant ($P > 0.05$) (Table 2). The SNK tests for the comparisons are shown in Table 2. Concentrations of Cd, Pb and Zn in the periostracum layer were significantly different ($P < 0.05$) from the concentrations in the other sections of the inner layer. Cu and Zn concentrations in the periostracum layer were significantly higher ($P < 0.05$) than in the other inner sections while Pb was the least accumulated metal in the periostracum layer being present there in a significantly lower concentration than in the inner shell layer. It is believed that there was incorporation of Cu and Zn through the surface of the periostracum. Moreover, the periostracum layer is more vulnerable and susceptible to the metal levels in the surrounding waters since it is the outermost layer of the shell. Apart from the direct adsorption pathway, the

TABLE 2
Student-Newman-Keuls (SNK) comparisons of Cu, Pb and Zn concentrations (means mg/g dry weight \pm standard error) in different sections in the shell of mussel *P. viridis*

Different sections Mean \pm SE Cu	Periostracum 12.02 \pm 0.31	Green-lipped 6.36 \pm 0.18	Ligament 6.20 \pm 0.20	Umbo 5.73 \pm 0.23	Ventral part 5.57 \pm 0.11	Dorsal part 5.55 \pm 0.19
Different sections Mean \pm SE Pb	Ligament 29.49 \pm 0.87	Green-lipped 28.65 \pm 1.44	Umbo 27.43 \pm 1.01	Ventral part 26.04 \pm 0.62	Dorsal part 25.96 \pm 0.83	Periostracum 10.87 \pm 0.75
Different sections Mean \pm SE Zn	Periostracum 13.78 \pm 1.80	Ligament 4.94 \pm 0.51	Green-lipped 3.76 \pm 0.30	Umbo 3.69 \pm 0.24	Dorsal part 2.90 \pm 0.44	Ventral part 2.68 \pm 0.10

Note: Means not differing significantly at $P < 0.05$ are indicated by a line under the corresponding values.

high concentrations of Cu and Zn in the periostracum layer could also be attributed to the biomineralization mechanism from the mantle of *P. viridis*. The periostracum layer's higher Cu and Zn levels might be due to the newly secreted extrapallial fluid which contained the components for biomineralization (Puentes *et al.*, 1996). This biomineralization composition might also contain heavy metals since the concentrations of Cu and Zn were higher in the soft tissue of *P. viridis*.

The inner shell layer exhibited insignificant differences ($p > 0.05$) in Cu, Pb and Zn concentrations amongst all sections in this shell layer although the metal distributions were not similar. The different distributions of chemical elements within the microstructure groups and mineralogical layers of the shell could be understood by looking at the chemical properties of trace elements in the shells (Carriker *et al.*, 1991). These chemical properties can provide a series of natural probes for the calcification system as well as fundamental information on the processes involved (Simkiss, 1983). Earlier, many researchers (Phillips, 1980; Wilbur and Saleuddin, 1983; Carriker *et al.*, 1991) had indicated that the heterogeneous distribution of metals in bivalve shell is a normal phenomenon. The complex polylayer of the shell structure in mussels further increased the potential for mineralogical and chemical variations.

Several authors (Wilbur and Saleuddin, 1983; Puentes *et al.*, 1996) had suggested the use of mollusc shells as biomonitoring materials. This is due to the fact that any trace metal which is actively incorporated into the shell matrix during shell growth must have been assimilated by the mussels (Wilbur and Saleuddin, 1983). According to Watson *et al.* (1995), some trace metals are incorporated into the shells of molluscs and barnacles through substitution of the calcium ion in the crystalline phase of the shell or they are associated with the organic matrix. The mineralogy and chemistry of the shell material secreted by organisms can vary with the environment of growth (Dodd, 1963). Al-Dabbas *et al.* (1984) suggested that shell composition is sufficiently sensitive to environmental variation that the environments can be distinguished within a limited water system.

CONCLUSION

The results showed tissue distribution of Cu, Pb and Zn in both the soft and hard (shell) tissues of *P. viridis*. The metal distribution in the different soft tissues could be due to different mechanisms of regulation, detoxification and physiological functions in each soft tissue. For example, specific organs such as the crystalline style, gill and byssus were identified to be potential biomonitoring organs for Cu, Pb and Zn. More ecotoxicological studies for the crystalline style, gill and byssus of *P. viridis* should be done. For the shell of *P. viridis*, the differential metal distributions found in the periostracum and in the inner layers (prismatic and nacreous) were mainly due to differences in the mineralogy and chemistry of the different shell layers. Our results indicated that the periostracum layer especially the green-lipped part of the mussel shell is a potential biomonitoring material for current metal levels in the environmental seawater. This is especially so for Cu and Zn since these metals could also adsorb onto the shell surface from the seawater. Experimental studies should be conducted for both the soft and hard tissues to confirm our hypothesis that they are suitable for use in the biomonitoring.

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Ectoparasites of *Rattus* sp from Petaling Jaya, Selangor, Malaysia

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ABSTRACT

Seven species of ectoparasites (Three Mites, two Sucking Lice, one Chewing Lice and one Dipteran) were recovered from the Brown Rat *Rattus norvegicus* (n = 10) and the Roof Rat *Rattus rattus* (n = 15) at the wet market of Jalan Othman, Section 3, Petaling Jaya, Selangor, Malaysia. The species collected in Brown Rat included *Echinolaelaps echidninus*, *Myobia* sp. and *Hoplopleura acanthopus* while those recovered from parasitized House Rat were *Echinolaelaps echidninus*, *Dermanyssus gallinae*, *Myobia* sp., *Hoplopleura acanthopus*, *Goniocotes gallinae* and one unidentified Anopluran and Dipteran species. Result of the investigation indicated that there was variation in the infestation and distribution of ectoparasite in both species. *Echinolaelaps echidninus* was the dominant ectoparasite species found on all rats captured (100% infested). The presence of *Dermanyssus gallinae* and *Goniocotes gallinae* was peculiar as these were normally found on avian species. As trapping was conducted near a market area, infestation by such ectoparasites could have originated from chickens as the original hosts. The potential of each ectoparasite in the transmission of zoonoses diseases was discussed.

INTRODUCTION

Most small mammal ectoparasite surveys within the state of Selangor, Malaysia, particularly in Bukit Lanjan and Air Hitam Forest Reserve were done in the late 80's (Shabrina *et al.*, 1989). Domestic rats, particularly those living in close association with man, play a major role in human health, welfare and economy. Their arthropod ectoparasites are important vectors of pathogenic organisms. Inherently, they are causative agents of many allergic disorders (Bakr *et al.*, 1996). No doubt, the increase in domestic rats population is followed by an increase in many zoonotic diseases, such as, scrub typhus (Lim *et al.*, 1980; Tanskul and Linthicum, 1999) and dermatitis (Rosen *et al.*, 2002). Due to the role of domestic rat ectoparasites such as chiggers, ticks and fleas as vectors of zoonotic pathogens, it is important to document host-parasite associations and infestation parameters for parasitic arthropods infesting domestic rats. The objective of the study was to examine the ectoparasite load and diversity of two common rat species, the roof rat

Rattus rattus and the brown rat *Rattus norvegicus* in urban areas in Malaysia.

MATERIALS AND METHODS

Study Site

The study was conducted at the wet market in Jalan Othman, Petaling Jaya, Selangor (latitude 3° 05 North and longitude 101° 38 East), Malaysia i.e. 10 km from Kuala Lumpur. It is a residential and commercial area. The map of the study site is shown in Fig. 1.

Rat Trapping

Rats were captured in standard commercial live traps baited with fresh prawns. Traps were placed on the floor at places where rats were noticeably active. Two to three traps were placed at each trap point. The trap layout is shown in Fig. 2. The traps were set at 2200 hours and inspected at 2400 hours. Trapping sessions were carried out for three consecutive nights i.e. from 21 March to 23 March 2002.



Fig. 1: Location of rat trapping site (X)

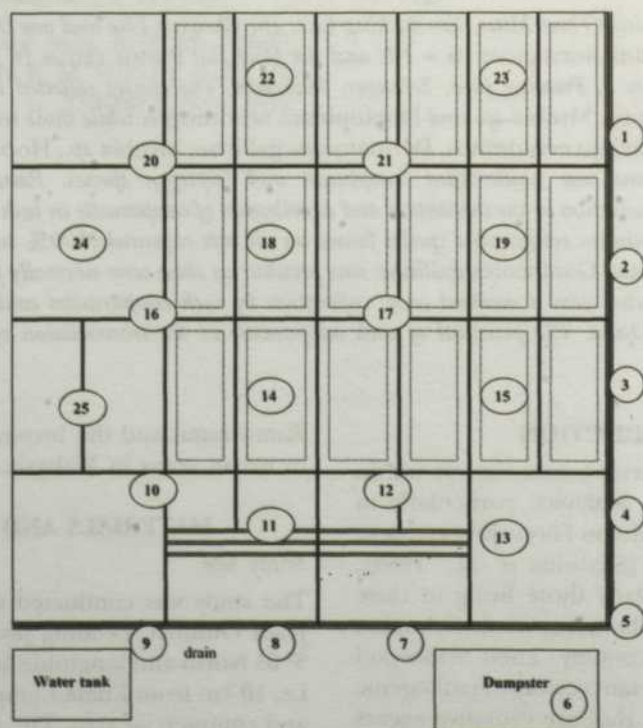


Fig. 2: Trap layout at the wet market of Jalan Othman, Petaling Jaya district of Selangor. Each number represents a trap point

Ectoparasite Removal and Collection

The captured rats were retained in the trap and brought to the laboratory, and killed by transferring into a cotton bag containing a piece of cotton soaked in chloroform. Once killed, the rats were removed from the cotton bag and brushed from tail to head, using a camel brush to remove any ectoparasites, which were collected on a white pan. The cotton bag was also

examined for the presence of ectoparasites that might have fallen from the animal when the latter were killed with chloroform. The ectoparasites were stored in vials containing 70% ethanol for preservation and subsequent microscopic examination (Durdin *et al.*, 2000). The contents of each vial were then observed under the light microscope and individual ectoparasites were carefully picked using a small

forceps, to avoid damaging the ectoparasites. The ectoparasites removed were transferred to fresh vials containing 70% alcohol for preservation and later identification (Durden, 1995; Durden *et al.*, 2000).

Ectoparasite Identification

Individual ectoparasites were mounted on slides in Hoyer's medium (50 ml distilled water, 30 g Arabic gum, 200 g chloral hydrate and 20 ml glycerine). Care was taken to avoid damaging the internal organs of the ectoparasites. Each mounted ectoparasite was cleaned by heating or immersing in lactophenol for at least 24 hours to remove the sclerotin that darkens the exoskeleton and prevent penetration of light, thus aiding in the identification. Mounted specimens were dried in the oven with temperatures between 40 °C to 50 °C for three days (Varma, 1993). Identification of ectoparasites was based on standard taxonomic keys and identification references (Kocan and Niec, 1975; Evans, 1992; Wall and Shearer, 2001). All ectoparasite specimens were sent to Acarology Division, Institute for Medical Research, Kuala Lumpur for further identification and confirmation of the ectoparasite species. (Dr. Ho Tzi Min, Inst. of Medical Research, Malaysia, Personal Communication.)

Rat Identification

Each of the rats captured was identified, labeled as male or female and measured. Identification of *Rattus rattus* and *Rattus norvegicus* was based on external features and measurements. The head and body length of *R. rattus* measures between 180 and 220 mm, tail length is between 185 and 240 mm, hind foot is less than 40 mm, and weighs between 80 to 300g (Tweedy, 1978; Medway, 1978). The head and body of *R. norvegicus* measures between 160 and 260 mm, tail length is between 170 to 230 mm, hind foot is more than 40 mm, and weighs between 200 and 485 g (Walker *et al.*, 1964; Tweedie, 1978; Medway, 1978). Rat species identification was used to compare the two species in terms of infestation levels, prevalence and relative densities of the ectoparasites collected.

Data Analysis

The following parameters as suggested by Durden (1995) were calculated for data analysis.

1. The percentage of rat infested with ectoparasites;

$$\text{Infestation (\%)} = \frac{\text{No of rats infested}}{\text{No. rats collected}} \times 100$$

2. The mean intensity of infestation;

$$\text{Mean Intensity} = \frac{\text{No. of ectoparasites collected}}{\text{No. of rats infested}}$$

3. The relative density i.e. mean no. of ectoparasites per rat host;

$$\text{Relative density} = \text{Infestation (\%)} \times \text{mean intensity}$$

RESULTS AND DISCUSSION

Twenty five domestic rats belonging to 2 species, Brown rat (*Rattus norvegicus*) and Roof rat (*Rattus rattus*) were trapped alive with a trap success rate of 31.2%. Table 1 lists the % infestation (percentage of hosts infested), mean intensity (mean per infested hosts) and relative density (mean per hosts) for each ectoparasite identified, actual number of ectoparasites and % composition (percentage of each ectoparasite from the total number of ectoparasites from each rat species). Seven species of ectoparasites (3 mites, 2 sucking lice, 1 chewing lice and 1 dipteran) were recovered from *R. norvegicus* and *R. rattus* and a total of 521 individual ectoparasites were collected from both rats. Seven species of ectoparasites (288 specimens) were collected from *R. rattus* compared to three species (233 specimens) collected from *R. norvegicus*. Telford *et al.* (1980) documented similar findings in terms of ectoparasite diversity between the two rat hosts.

Echinolaelaps echidninus was common to both *R. norvegicus* and *R. rattus* bearing the highest infestation rate i.e. 100% and 93.3%, respectively. *E. echidninus* is a well documented ectoparasite of rats (Botelho and Linardi, 1996; El Deeb *et al.*, 1999). Table 1 also shows that % composition of *E. echidninus* was higher in *R. norvegicus* (92 %) than *R. rattus* (51 %). Telford *et al.* (1980) found that *E. echidninus* accounted for 75 % of the ectoparasites recovered from *R. rattus*. According to Schmidt and Roberts (2000) and Marquardt *et al.* (2000), *E. echidninus* has the potential to spread the protozoan *Hepatozoon*

TABLE 1
Ectoparasites recovered from 25 domestic rats at the wet market of Jalan Othman,
Section 3, Petaling Jaya, Selangor, 2002

Host Species	Ectoparasites*	I (%)	MI	RD	n	C(%)**
Brown rat, <i>Rattus norvegicus</i> n = 10 (2 males, 8 females)	Mites					
	<i>Echinolaelaps echidninus</i>	100	21.4	21.4	214	92
	<i>Myobia</i> sp.	30	3.7	1.1	11	5
	Sucking Lice (Order: Anoplura)					
	<i>Hoplopleura acanthopus</i>	50	1.6	0.8	8	3
Roof rat, <i>Rattus rattus</i> n = 15 (8 males, 7 females)	Mites					
	<i>Echinolaelaps echidninus</i>	93	10.4	9.7	146	51
	<i>Dermanyssus gallinae</i>	40	14.7	5.9	2	0.6
	<i>Myobia</i> sp.	13	1.0	0.1	30	10
	Sucking Lice (Order: Anoplura)					
	<i>Hoplopleura acanthopus</i>	60	3.3	2.0	88	31
	<i>Antarctopthirus</i> sp.	33	3.4	1.1	17	6
	Chewing Lice (Order: Mallophaga)					
	<i>Goniocotes gallinae</i>	13	2.0	0.3	4	1
	Flies					
Diptera (unidentified)	7	1.0	0.1	1	0.3	

* For each ectoparasite species, infestation parameters listed are % infested, I; mean intensity (mean per infested host), MI; relative density (mean per host), RD; actual no. of ectoparasites, n; and % composition, C.

** T-test indicates that the ectoparasite composition was not significantly different between the two rat species, $t = 0.002$, ns.

muris in rat populations, but thus far, there is no evidence that it infects humans (Schmidt dan Roberts, 2000).

Myobia sp. and *Hoplopleura acanthopus* were also recovered from both rat hosts. The presence of prostigmatid mites, *Myobia* sp. is a matter of interest. According to Hirst (1922), a number of mites from the subfamily Cheyletinae were not dependent on their host and are predators of other mites such as Tyroglyphid mites or other small arthropods, but are sometimes, also parasitic.

Ninety six specimens or 18.4 % of all ectoparasites recorded were the sucking lice, *Hoplopleura acanthopus* (Order: Anoplura) (Table 1). *H. acanthopus* was found on both host species but with higher infestation and percentage composition on *R. rattus* compared to *R. norvegicus*. Hopkins (1949) stated that the genus *Rattus* is often infested with *Hoplopleura* sp. and *Polyplax* sp. King *et al.* (1980) reported that 25 % of *Rattus norvegicus* and 20% of *R. rattus* examined were infested with *Polyplax* sp. and *Hoplopleura* sp. These lice may play a

supplementary role in the infection of murine typhus in rat populations (King *et al.*, 1980).

The occurrence of *Dermanyssus gallinae* and *Goniocotes gallinae* were peculiar, which are normally found on avian species. Close proximity between rat populations and chickens is a plausible explanation. This phenomenon was also reported by Bakr *et al.* (1995) wherein *D. gallinae* found on rodents were in close association with domestic animals. *D. gallinae* has been known to infest domestic chickens and turkey and wild birds such as pigeons, sparrows and starlings. (Varma, 1993). *D. gallinae* has been suggested as a vector for the St Louis encephalitis arbovirus (Varma, 1993). This was substantiated by Mehlhorn (2001), who associated *D. gallinae* with the spread of St. Louis encephalitis virus and anemia on chickens. Regan *et al.* (1987) claimed that *D. gallinae* is a potential vector for the eastern and western equine encephalomyelitis virus. According to Kirkwood (1967), *D. gallinae* can cause anemia, leading to lower egg production in domestic chickens and

birds and may cause death of the host species. *D. gallinae* also poses a potential health hazard to workers in poultry pens and chicken farms (Hoffman, 1987). According to Bowman *et al.* (2002), *D. gallinae* is the only chicken mite that can cause dermatitis to humans, particularly when there is an absence of avian hosts, nearby poultry pens and chicken processing units.

Goniocotes gallinae commonly known as chicken lice was only found on *R. rattus*. *R. rattus* is not a usual host for *G. gallinae* which may become infected with the latter when the normal host dies. Since the study site is a wet market where chickens were slaughtered, it is most likely the ectoparasite looks for an alternative host i.e. *Rattus rattus* that are in close proximity to the chicken host.

The presence of *G. gallinae* and *D. gallinae* on rat host in this study is a new documentation on host-parasite interaction. A similar host-parasite interaction involving domestic cats and *D. gallinae* has been reported by Muller *et al.* (1983) and Grant (1985; 1989). Most of the cases of *D. gallinae* infestation of domestic cats can be related to the close association between the former and chicken farms (Bowman *et al.*, 2002). This supports the present study which indicates a close association between domestic chicken *Gallus gallus* and the roof rat, *Rattus rattus*, in terms of host parasite interaction.

Xenopsylla cheopis, a vector of the dreadful bubonic plague was absent from the sampled rat hosts. Any epidermic zoonoses, if present, may be restricted within the rat population as indicated by the high infestation of *E. echidninus* in the study area.

In terms of ectoparasitic composition, the two rat species were not different (T-test; $t = 0.002$, ns), although *R. rattus* carries double the number of ectoparasite species, i.e. six compared to three in *R. norvegicus*. In terms of ectoparasite composition, *R. norvegicus* recorded 92% *Echinolaelaps echidninus* compared to 51% in *R. rattus*. The latter also recorded 30% *Hoplopleura acanthopus*. This may reflect the different ecology and foraging habits of the two commensal rats.

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Multiple Satellite Detection of Hotspots in Peninsular Malaysia during February and March of 2002

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ABSTRACT

A comparison of the spatial distribution of hotspots detected in Peninsular Malaysia during the short spell of dry conditions that occurred in February and March of 2002 from three different types of satellite programmes such as National Oceanic Atmospheric Administration (NOAA), the Terra Moderate Resolution Imaging Spectroradiometer (MODIS) and Defense Meteorological Satellite Programme (DMSP) satellites was investigated. This period was considered unusual compared to other years, due to the high level of burning activities and the early occurrence of haze compared to the usual burning period from July to September. The spatial patterns of the locations of hotspots detected from these multiple sources were analyzed, although they differed in the overpass times, algorithms, resolutions and cloud coverage that rendered dissimilarities in the hotspots detected. Visual interpretation on the patterns of hotspots showed that Pahang, Johor and Kedah states of Peninsular Malaysia recorded the largest number of hotspots.

INTRODUCTION

The notable 1997 regional transboundary haze crisis that hit the Southeast Asian region including countries such as Singapore, Brunei, Malaysia and Indonesia resulted from the destruction of approximately 10 million hectares of natural forest in Indonesia and incurred an economic loss of over US\$10 billion (Boyd, 2002). The problem has not abated, since the recurrence of transboundary haze due to vegetation burning on a large scale has continued since 1997. For the early burning season of February to March 2002, approximately 10,906 hectares of plantations and protected forest reserves were destroyed, which include hundreds of hectares of peat land in Bengkalis, Riau on the island of Sumatra (*The Jakarta Post*, 2002). Most of the fires were left unattended due to the lack of personnel in the local forest department in Sumatra and the inaccessibility of the remote areas. Only surface fires were tackled, while undergrowth fires were left smoldering, as they were difficult to extinguish. The haze in Riau affected the local air quality where visibility was reduced to 20 m in Mantau, Bengkalis (*The Jakarta Post*, 2002).

Although the forest reserves in Malaysia are not as plentiful as in Indonesia, the burning activities that occur can still affect the local environment (Abdullah *et al.*, 2002; Maarof, 2002; Musa and Parlan, 2002; Gawan, 2001; Sangaran, 2001). A total of 543 hectares of land was burnt in Sepang in the state of Selangor in Peninsular Malaysia during February and March of 2002 (*Berita Harian*, 2000a). Approximately 400 hectares of agriculture land and the fringe of forests were burnt by smallholder farmers near Sepang and Kuala Selangor on 15th February and over one hundred hectares of peat land burnt that was strenuous to douse took place during the dry conditions in February (*Utusan Malaysia*, 2002a). In total, 8000 hectares of forest area throughout Peninsular Malaysia was burnt, mostly in the states of Selangor and Pahang (*Utusan Malaysia*, 2002b). Amongst the areas involved were also oil palm plantations of over 3115 hectares near Pekan, Pahang (*Utusan Malaysia*, 2002c).

With the advance of technology, remotely sensed satellites can disclose the active fire counts and spatial areas of burning on a daily basis. It can provide information on the monitoring of

different aspects of fires, such as the areas at risk to fires, fire capabilities, burned area, and active fires, smoke plumes or trace gases (WWW1). Data from multiple satellites can be compared to identify the locations of ground truth burning. Remote sensing offers a cost-effective approach to attain an inclusive observation of fire activities in near-real time, particularly over remote, unpopulated areas (WWW2).

Large-scale fire events have been detected by a variety of satellite images that comprise various resolutions and multi-temporal intervals such as SPOT (Liew *et al.*, 1998), National Ocean Atmospheric Administration (NOAA) Advanced Very High Resolution Radiometer (AVHRR) (Wooster *et al.*, 1998), Along Track Scanning Radiometer (ATSR) hotspot data (Siegert *et al.*, 2000), Landsat TM (Lim *et al.*, 2004) or the European Radar Satellite-2 Synthetic Aperture Radar (ERS-2 SAR) (Buongiorno *et al.*, 1997). Algorithms were also constructed to detect burn scar mapping using moderate spatial resolution satellites (Roy *et al.*, 2002a). Burnt scars using the output of active-fire algorithms have also been investigated for global application from NOAA images (Roy *et al.*, 1999).

The objective of this study was a preliminary comparison of the hotspots detected by various satellites such as NOAA AVHRR, Terra Moderate Resolution Imaging Spectroradiometer (MODIS) and Defence Meteorological Satellite Programme (DMSP) Optical Line Scan (OLS).

The results of this study can help the Malaysian authorities and managers of fires such as the Fire Department, Police and the Department of Environment in evaluating the trends of the burning and to adopt policies that may control or manage future fires.

MATERIALS AND METHODS

Spatial distribution analysis was utilized to establish the distribution patterns of the active fires throughout the day. The area of interest is the Southeast Asian region, with emphasis on Peninsular Malaysia. The period investigated was for the short dry episode in February and March of 2002. The NOAA AVHRR hotspot data was obtained from the Forest Fire Prevention Monitoring Project II (FFPMPII) project that is based in Indonesia. The project is supported by Japan International Cooperation Agency (JICA) and is a collaborative effort with the Department of Forestry, Indonesia. The daily hotspot data is

hosted at their website for easy access to the public. The MODIS hotspot data was obtained from the rapid-fire dataset, while the DMSP data was obtained from the Asia-Pacific Network for Disaster Monitoring using Earth Observation Satellite (ANDES) project supported by the Japan Science and Technology Corporation. ANDES is developed as a near-real time operational information system that monitors and mitigate agriculture and forest fires disasters (Sawada, 2002). The data is archived for public use and transferred to East and Southeast Asian countries.

Terra MODIS acquires data in 36 spectral bands that covers the visible, near infrared, short wave infrared, medium wave infrared and thermal infrared (Kaufman *et al.*, 1998). MODIS comprise of 16 thermal bands with high saturation levels suitable for detecting hotspots (Chen *et al.*, 2001). The resolution of the thermal anomalies product is 1 km. The overpass time of Terra is at approximately 1000 am local time. Active fires are detected from the 4 and 11 μm bands, with high saturation temperatures of approximately 450K and 400K, respectively (WWW2). The active fires are detected through the fixed-threshold and a contextual algorithm (Giglio *et al.*, 2003), with different criteria for daytime and nighttime imageries. Each pixel of the MODIS swath is assigned the classes of missing data, cloud, water, non-fire, fire or unknown (WWW3). The performance of the MODIS product is continually examined through quality assessment and validation activities (Roy *et al.*, 2002b).

Fire detection from the AVHRR channel is exploited at the middle thermal infrared channel at approximately 3.7 μm that is sensitive to objects emitting thermal energy at high temperatures of more than 200°C (WWW2). An existence of fire or hotspot lies within the spatial area of 1.1 km², without any information on the size, numbers or intensities of fires or burnt area (WWW4). Its moderate calibration and low saturation temperature render the hotspots detected by NOAA AVHRR to inherit many false alarms and a tendency of an underestimation of fire areas or over estimation of the number of fires (Chen *et al.*, 2001).

The OLS is an oscillating scan radiometer designed for cloud imaging with the visible near infrared and thermal infrared bands initially utilized to detect clouds using moonlight, gas flares, lightning, city lights and fires (WWW4). The night time passes occur between 2030 and

2130 local time (Elvidge *et al.*, 2001). The processing algorithms and extractions of fires are described in detail by Elvidge *et al.* (1997). Its spatial resolution of 2.7 km and applications of light emission from night time fires makes this a relatively coarser image than either the NOAA or MODIS images.

Spatial analysis was performed on the active fires detected by the three satellites. Simple centrographic statistics such as the central locations and standard distances or the standard distance deviation were initially calculated. The standard distance deviation (SDD) provides information on the dispersion of the active fire counts around the mean centre. The first-order properties of spatial distribution was carried out where parameters such as the autocorrelation Moran and Geary's C indices can resolve if the hotspots detected by the three satellites exhibit strong correlation tendencies with their neighbours. The neighbourhood patterns and clusters within the overall distribution were then subjected to a second-order spatial analysis, which is a measure of the mean nearest neighbour distance. The nearest neighbour index was calculated to determine if the spread of hotspots were clustered, dispersed or randomly distributed.

Further filtering was performed to identify different groups of clusters of hotspots within the Peninsular Malaysia. The technique employed was the nearest neighbour hierarchical spatial clustering routine that identifies groups of objects that are spatially closer than it would be expected on the basis of chance (Levine, 2002). This technique utilizes a nearest neighbour method that specifies a threshold distance and compares that to the distances of all pairs of points. A minimum of five points per cluster was selected as the cluster threshold for all the group of clusters. Clustering was performed on several levels, where only the clusters that fit the five points limit passes on to the next level.

RESULTS

Weather Conditions

The dry conditions of the El Nino phenomenon during the 1997 haze in Southeast Asia was one of the reasons that aggravated the haze conditions from the large scale biomass burning in Indonesia. The NOAA Climate Prediction Center (NOAA Magazine, 2002) that monitored the Pacific Ocean surface temperatures found an

increase of 2°C in February 2002, indicating a progression of an El Nino condition, forecasted as a moderate event that continued until early 2003. This was obtained from evidence of NOAA's global climate monitoring system that consist of data from satellites and moored buoys over the equatorial Pacific Ocean with the purpose of providing real-time atmospheric and oceanic data. Fig. 1 shows the South Oscillation Index (SOI) over the equatorial Pacific Ocean and the Indonesia sea level pressure anomaly (SLPA) from 1997 to early 2003. The SOI and Indonesian SLPA were in opposite phases during the El Nino periods. The 1997 El Nino was exhibited by negative SOIs compared to the positive magnitudes of the Indonesian SLPA. The starting point of the moderate El Nino began in March 2002, exhibited by weak SOI magnitudes of approximately -2, in contrast to the strongly negative SOI that peaked to -6 in 1997. Thus, the sea surface temperature increase over the Pacific Ocean, a precursor to the El Nino presence was found in February and revealed by the SOI in March.

On a local scale, Peninsular Malaysia was affected by the dry conditions in February and March 2002. It is not clear whether the occurrence of dry condition over Peninsular Malaysia was a precursor to the moderate El Nino event of 2002. The El Nino and the La Nina episodes are extreme deviations of the Tropical Biennial Oscillation theory postulated by Meehl (1997) that encompass the coupled land-ocean-atmosphere climate system. In this climate system, the transition to a relatively strong or weak Asian or Australian monsoon is caused by a variety of large and regional-scale conditions in the seasons preceding the monsoon established by the coupled air-sea interactions of the year before (Meehl and Arblaster, 2002a). Transitions from March to May and from June to September are found to establish the coupled atmosphere-land-ocean interactions for the following year, with an opposite transition in the subsequent year (Meehl and Arblaster, 2002b).

Table 1 shows the list of 18 stations in P. Malaysia that exhibit negative deviations of rainfall from the normal, with an average monthly negative deviation of 67% and a monthly total mean of 40 mm in February. Stations such as Chuping, Pulau Langkawi, Alor Star and Bayan Lepas represent the northwestern region of Peninsular Malaysia, while stations Kota Bharu

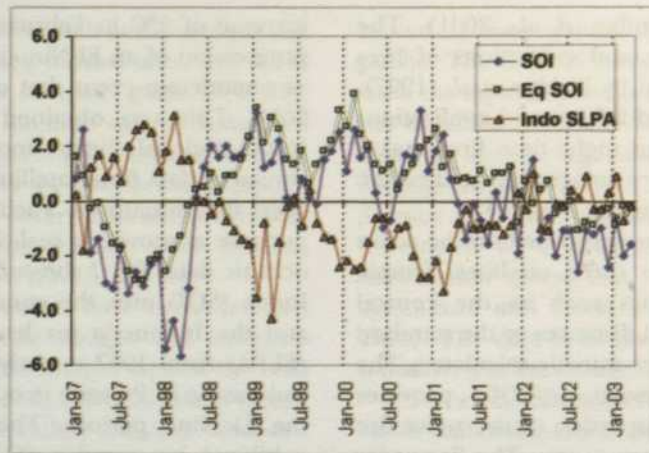


Fig. 1: The Southern Oscillation Index and the Indonesian sea level pressure anomaly from 1997 to early 2003

through to Mersing represent the eastern region. Conditions are slightly wetter in March, where most of the southern stations exhibit negative anomalies of magnitudes below 30%, compared to the northern part of the peninsula in the states of Perlis, Kedah and Kelantan that exhibit negative deviations of more than 60% from the normal, representing very dry conditions. The trend in rainfall patterns was similar to that in February, where the northern and northwestern states received far less rainfall than either the western coast or southern region of Peninsular Malaysia. The average rainfall for the peninsula of 124 mm in March was approximately 24 % below normal.

The temperature deviations from climatological normal showed in Table 1 also illustrate that most of the stations over the Malaysian peninsula exhibit a warming tendency of approximately 20% above normal. The maximum temperatures recorded reached as high as 36.4°C in February and 36.6°C in March at the urban location of Petaling Jaya in Selangor (*Utusan Malaysia*, 2002b). The mean deviations for most of the stations in P. Malaysia were positive, at values of 0.53 and 0.84 in February and March, respectively. Weather parameters such as temperature, evaporation and solar radiation all illustrate above normal conditions, whilst rainfall exhibited negative anomalies, indicating that the Malaysian peninsula was drier and warmer than normal, in which these conditions were conducive to burning where dry vegetation materials were prone to fires and

produced smoke easily. Therefore, the burning activities over Peninsular Malaysia were exacerbated by the drier conditions.

Biomass Burning Activities Detected from Multiple Satellite Sources

The hotspots detected by the NOAA and MODIS satellites over Sumatra and Peninsular Malaysia from February and March 2002 indicate that the land clearing activities in Sumatra was dynamic (Fig. 2). The highest number of hotspots occurred in early February when the daily number peaked at 1,230 and 1,184 hotspots on the 11th and 12th March, respectively. Most of the hotspots on 11th March were identified in Sumatra, with a total of 875 hotspots in Riau, 45 in North Sumatra and 188 in Peninsular Malaysia. A total of 597 hotspots were detected in Riau, in contrast to 198 hotspots over Peninsular Malaysia on the following day, indicating vigorous burning activity over the two areas during these two days. The number of NOAA AVHRR hotspots detected by the Meteorological Services of Singapore (MSS) was more conservative than the FFPMPIL, with the highest hotspots of 550 on 10th March. The number of hotspots registered on the 11th and 12th March was 360 and 310, respectively. Although both centres used the same AVHRR data, their algorithms utilized were different. The mid-morning fires detected by MODIS were the most conservative, with the maximum hotspots occurring on 8th March, totaling 159 hotspots for both Peninsular Malaysia and Sumatra.

MULTIPLE SATELLITE DETECTION OF HOTSPOTS IN PENINSULAR MALAYSIA

TABLE 1
The anomalies of rainfall, solar radiation and evaporation in Peninsular Malaysia from February to March 2002

Stations	% Anomaly Rainfall (mm)		Temperature (°C)		Deviant from Normal Solar Radiation (MJ/m ²)		Evaporation (mm/day)	
	Months							
	Feb	Mar	Feb	Mar	Feb	Mar	Feb	Mar
Chuping	-97	-27	0	1	12	9	17	18
P. Langkawi	-83	-79	1	1	7	6	17	9
Alor Star	-63	-49	1	1	4	4	19	21
Bayan Lepas	-59	-45	1	2	10	7	4	8
Ipoh	-38	-31	1	1	5	3	10	8
Cameron Highlands	-94	-62	0	0	10	9	12	-20
Sitiawan	-23	-1	1	1	6	9	17	11
Subang	-13	-41	2	2	2	-2	11	4
Kluang	-76	-4	0	1	4	8	8	18
Senai	-66	-4	0	0	-5	3	11	24
Kota Bharu	-65	-27	0	0	0	0	9	9
Kuala Krai	-80	-16	0	1	9	12	5	7
Kuala Trengganu	-91	-29	0	0	-5	-3	-4	6
Kuantan	-94	-10	1	1	13	11	18	21
Batu Embun	-81	35	0	1	11	14	6	16
Temerloh	-94	-71	0	1	4	-4	8	9
Muadzam Shah	-57	-49	0	1	5	11	-3	10
Mersing	-26	-46	1	1	10	10	4	-5
Average	-67	-31	0	1	6	6	9	10

Source: Malaysian Meteorological Services (2002)

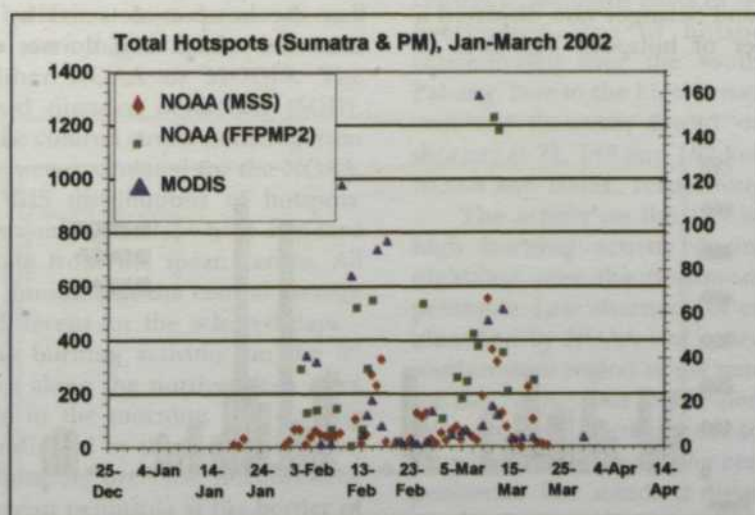


Fig. 2: The activity of burning over Sumatra and Peninsular Malaysia as detected by three types satellites from February to March of 2002

The activity of burning in Sumatra was of a larger scale than in Peninsular Malaysia due to her large-scale development programme of converting vegetated areas into oil palm plantations. This is reflected by the total of only 498 hotspots in February detected by NOAA satellite (Fig. 3). Two consecutive days with the highest activity of burning were on the 14th February, with 111 hotspots, followed by the next day that recorded a total of 130 hotspots. In contrast, the DMSP satellite detected a total of 1,044 hotspots during February. Another similar pattern of consecutive days of high hotspots was detected, on the 24th, with a total of 145 hotspots, followed by 198 hotspots on the next day. No hotspots were detected for a total of eight days during this month, which were the 6th, 15th, 16th, 17th, 19th, 20th, and 26th to 29th. The highest single day total hotspots of 85 detected by MODIS were on the 18th February, followed by 81 hotspots detected on the 9th February and 69 hotspots on the 11th. There were six days in March where no hotspots were reported by the FFPMPPI project on the 2nd, 3rd, 10th, 15th, 16th and 17th. The latter missing observations were due to the break in record when their office was relocated from Bogor to their present location in Jakarta. Regrettably, vital information was lost when the transboundary haze was at its peak from the 15th to 17th of March.

The state of Pahang exhibited the highest number of hotspots detected at 601, followed by Johore at 365 and Kedah at 235. Terengganu, Negeri Sembilan and Selangor also displayed a substantial number of hotspots. The fires in

Selangor received much media coverage due to its close proximity to the capital, Kuala Lumpur, but there was prominent burning of the agricultural waste in February particularly over the northern states such as in Kedah. The burning of the peatland in Pahang emerged as near continuous throughout the two months, with prominence in March 2002.

The trend of burning shown in the two months emerged as if the burning activities only occurred on specific days. One of the factors that hinder continuous detection is the presence of clouds, which consists of thick cumulonimbus clouds characteristic of equatorial regions. Only the nighttime hotspots detected by DMSP showed the near continuous activity of burning over the Malaysian peninsula, although this too was influenced by the presence of clouds. The clearing of agriculture land through burning by the local farmers who prefer to burn during the afternoon and evening is a clear disadvantage for the monitoring or detection of hotspots by the Terra MODIS satellite that has a morning overpass time.

There exists a weak relationship between the hotspots from the three satellites. The correlation coefficient between the hotspots detected by NOAA and DMSP was moderate with a coefficient of 0.4, compared to the lower coefficient of 0.28 between NOAA and MODIS as shown in Table 2. The correlation between MODIS and DMSP was much lower with a coefficient of 0.17. ANOVA analysis confirmed that the hotspots detected by three satellites were not related and do not come from the

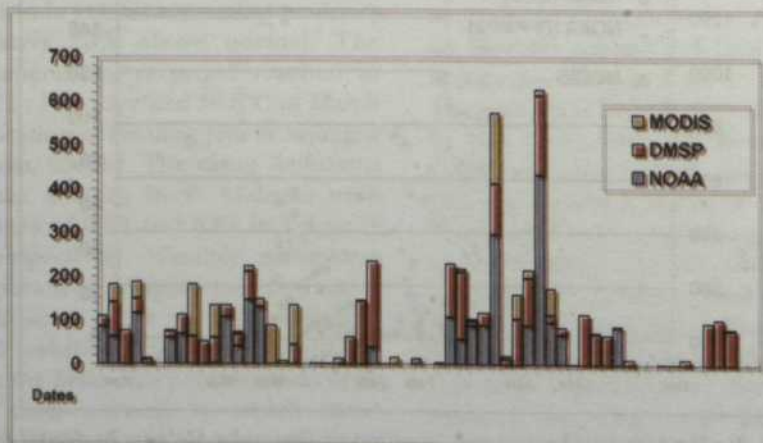


Fig. 3: Hotspots in Peninsular Malaysia as detected by three different satellites such as the AVHRR NOAA, DMSP and MODIS from 1-February to 30 March 2002

same population. This is to be expected as the over pass time of each satellite is different. Terra MODIS detects the morning observation, while NOAA detects the late afternoon burning and DMSP the nighttime fire activity.

TABLE 2
Correlation coefficients between hotspots of the three satellites

Correlation	NOAA	DMSP	MODIS
NOAA	1.000	0.399	0.282
DMSP	0.399	1.000	0.171
MODIS	0.282	0.171	1.000
N cases	59	59	59

Spatial Analysis of Hotspots

Visual interpretation of the spatial distributions of hotspots detected by the three satellites was limited, as comparison when all the satellites were present on the same day could not be performed for the whole month. Only four days experienced this occurrence which were on the 6th, 8th, 11th and 13th March 2002.

Fig. 4 shows the superimposed hotspots from the three satellites. Each of the four days illustrated different patterns of fire distributions that were variable spatially and temporally. The total number of hotspots from the three satellites were moderate on the 6th March, compared to the higher burning activities on the 8th and the 11th March. On both these days, the numbers of DMSP hotspots were higher than the ones detected by either NOAA or MODIS. The different standard distance deviations (SDD), represented by the colored circles radiating from the mean centre were calculated for the NOAA, DMSP and MODIS distributions of hotspots, with their corresponding circles whose standard distances originate from the mean centre. All the four panels showed that the central average locations were different for the selected days.

Much of the burning activities on the 6th March took place along the northwestern coast of the peninsula in the morning and evening time. This was indicated by the central location of the NOAA hotspots over the northwestern part of the Malaysian peninsula at the border of Kelantan-Perak (Table 3). The SDD of 177 km contain most of the hotspots detected over the northern and northwestern states, including approximately 68% of the total NOAA hotspots.

Nighttime burning was also concentrated over the northwestern region of the peninsula, with another cluster of high burning over the central state of Pahang. However, the SDD for the hotspots from the DMSP satellite was large at 206 km due to the low number of hotspots totalling 35 compared to the higher density of NOAA hotspots that totaled 125. The SDD for the MODIS hotspots also coincided with the SDD for the NOAA satellite and covered a part of the DMSP circle of dispersed distribution. This reveals that the distribution of hotspots detected from the three satellites all concentrate over the northern to northwestern part of the peninsula.

The DMSP satellite detected high burning in the central and southern states of Pahang, Negeri Sembilan and Johor on the 8th at nighttime in contrast to the occurrences over the eastern part of the peninsula. This differed from the clustered distribution of the NOAA hotspots in the southern states of Johor, and eastern and central states of Terengganu and Pahang. Hotspots detected by MODIS were also detected in Pahang, overlying the area detected by NOAA and DMSP particularly over the central eastern coast of the peninsula. This is the location of peat forest that burned for several days as reported in the local media (*Utusan Malaysia*, 2002b; 2002c). The SDDs of burning activities as detected by NOAA, MODIS and DMSP were superimposed over the same area, covering the central to southern parts of the peninsula. The overlapped area of hotspots activity was concentrated over the southeastern state of Pahang. Due to the high density of hotspots, the standard distances found on this day were shorter, at 71, 143 and 162 km, for the MODIS, NOAA and DMSP, respectively.

The activity on the 11th March still showed high burning activity during morning and nighttime over the northwestern coast of the peninsula. Late afternoon or evening burning as identified by NOAA was concentrated over the southwestern region of the peninsula. The SDDs for the NOAA and DMSP derived hotspots for this day also overlapped each other, mainly covering a large area in the central region of the peninsula. The standard distances displayed by the distribution of hotspots from the two satellites were larger than on the 8th March, due to the dispersion of hotspots that were clustered over the northwestern and southwestern region of

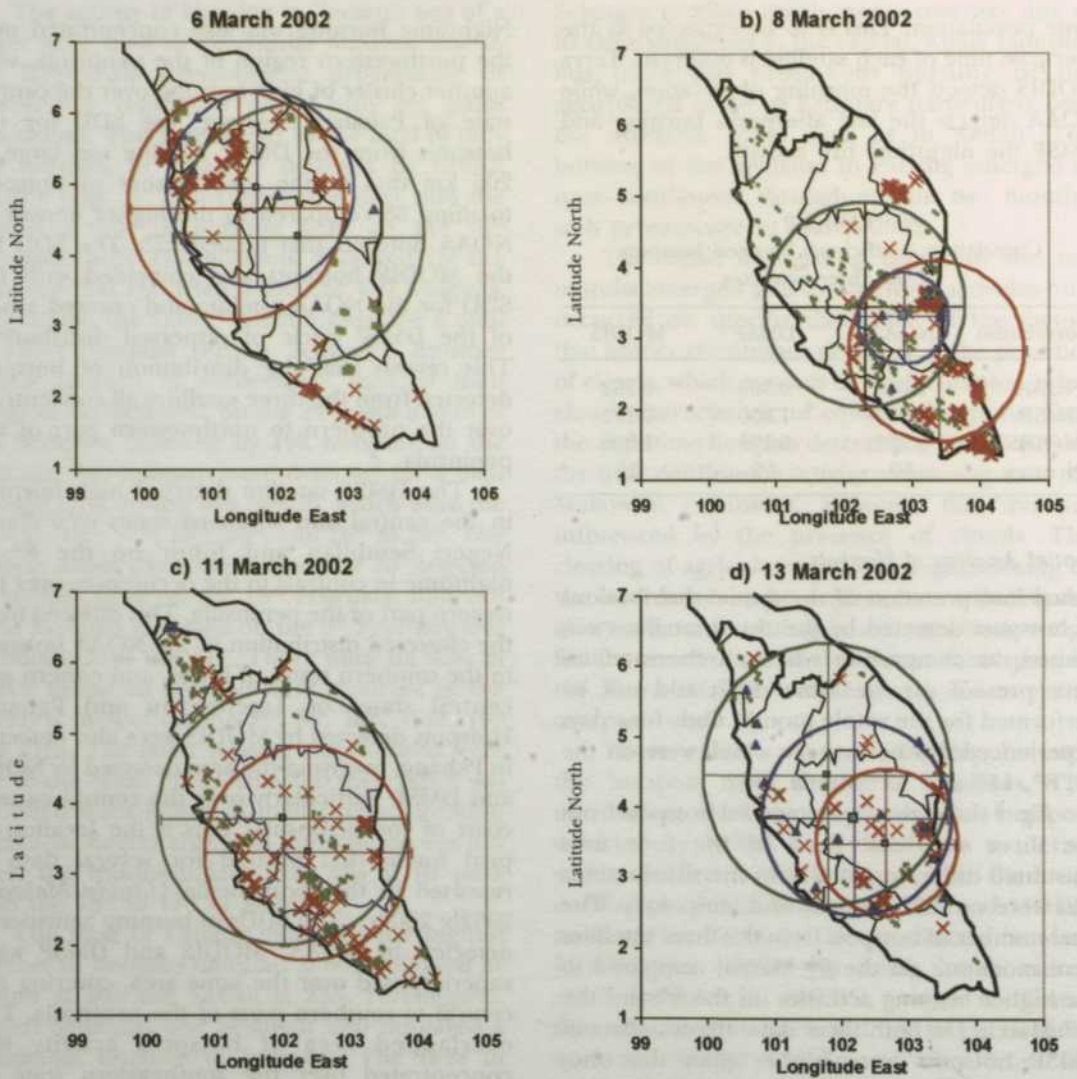


Fig. 4: The distribution of hotspots on four selected days in March 2002. The x symbol represents the NOAA data, Δ represents the MODIS hotspots and \blacksquare represents the DMSP data. The respective central locations (■) and standard distances are plotted on four selected days in March 2002

the peninsula. MODIS only detected two hotspots over Perlis, located in the northwestern peninsula. The moderate burning activity in the morning of 13th March was located along the northern part of the western coast and over the central part of the peninsula. Evening burning, detected by NOAA was mainly found over the central region of the peninsula. This is reflected by the overlapped areas of SDDs for each of the satellites, which was located mainly in the central state of Pahang

Table 3 shows that for the selected four days, all the Moran and Geary C indices were positive but less than one, indicating the hotspots

were mostly clustered rather than dispersed. The NOAA hotspots consistently exhibited higher values of Moran I, with magnitudes of more than 0.61 for all the four selected days. This suggests that the active daytime hotspots were more spatially correlated or clustered compared to either the morning or nighttime burning activities. This trend was also displayed by the MODIS hotspots, which exhibited moderately high positive Moran indices. This is in contrast to the DMSP hotspots that exhibit lower magnitudes of indices compared to either the MODIS or NOAA satellites, implying that there was less spatial correlation or less clustering

compared to either the morning or evening time burning. The second-order spatial analysis that defines the local properties of distribution was investigated, to identify the neighbourhood patterns and clusters within the overall distribution (Table 4). The mean nearest neighbour distance (MNND) varied for different days. The MNND was large on the 6th for the NOAA hotspots compared to the hotspots from other satellites. Only the distribution of hotspots detected by MODIS exhibited a MNND index of more than 1, indicating random dispersion on the 13th March 2002 and a dispersed distribution on the 6th March. This was due to the small number of hotspots detected that are widely dispersed from one another. The nearest neighbour hierarchical (NNH) cluster analysis highlights the difference between the daytime and nighttime spatial variability for the four selected days. The spatial patterns do not indicate that the first-order clusters of daytime hotspots were followed by the first-order clusters of nighttime burning activities as depicted by the NNH1 notation in Fig. 5. The placements of the clusters of daytime and nighttime burning were different on all the selected four days. Only two of the second-order clusters were exhibited by the hotspots from the NOAA satellite, as shown on 6 and 13 March. They were grouped from the eight individual first-order clusters on 6

March and from 4 individual first-order clusters on 13 March (Fig. 5). No second-order clusters were found from the nine first-order clusters or the sixteen first-order clusters for the DMSP derived hotspots on 11 March. This was due to the hotspots that were dispersed over large distances throughout the peninsula.

However, they highlight the information on the different concentrations of burning activities that may be useful to fire managers such as the local Fire Department as a contingency plan to strategically tackle the concentrated clusters of fires if resources are limited. In this study, five hotspots per cluster were chosen as the threshold criteria. However, the threshold counts could be increased to limit or reduce the groups of clusters within a selected region.

An index of dissimilarity that functions as an indicator of relative change of concentration was presented to study the difference between the paired percentages from the hotspot distributions detected by the satellites. The dissimilarity indices between NOAA and DMSP for the four days investigated were moderate over the states in Peninsular Malaysia, indicating the patterns of distributions were approximately 30% to 70% different over the various states as shown in Table 5. The highest dissimilarity of 69% occurred on the 6th March. The spatial distribution shown in Fig. 4a demonstrate that

TABLE 3
The centographic statistics for the NOAA, DMSP and MODIS satellites

Satellite	Mean Centre		State	Standard distance (km)	Moran I	Geary C
	Longitude °E	Latitude °N				
a) 06 Mar 2002						
NOAA	101.09	5.01	Perak	113.26	0.61	0.33
DMSP	102.31	4.07	Pahang	203.58	0.65	0.27
MODIS	101.42	5.15	Perak	167.70	0.15	0.89
b) 08 Mar 2002						
NOAA	103.57	2.41	Johor	117.73	0.73	0.17
DMSP	102.45	3.24	Pahang	130.23	0.34	0.50
MODIS	102.99	3.45	Pahang	64.96	0.55	0.10
c) 11 Mar 2002						
NOAA	102.70	3.02	Pahang	134.23	0.63	0.22
DMSP	102.13	3.53	Pahang	214.11	0.41	0.52
MODIS	100.38	6.51	Kedah	7.66	NA	NA
d) 13 Mar 2002						
NOAA	102.87	3.45	Pahang	77.22	0.63	0.19
DMSP	101.74	4.52	Pahang	203.26	0.51	0.44
MODIS	102.50	3.69	Pahang	136.15	0.85	0.17

NA indicates data is not available.

TABLE 4
The second-order local properties of neighbourhood hotspot patterns of the three satellites

Date	Satellites	Mean Nearest Neighbour Distance	Expected Neighbour Distance	Nearest Neighbour Index	Distribution
06 Mar 02	NOAA	5.73 km	20.10 km	0.28	cluster
06 Mar 02	DMSP	3.64 km	30.23 km	0.12	cluster
06 Mar 02	MODIS	101.53km	6.17 km	1.65	disperse
08 Mar 02	NOAA	2.25 km	10.35 km	0.22	cluster
08 Mar 02	DMSP	3.01 km	10.05 km	0.30	cluster
08 Mar 02	MODIS	17.74 km	46.84 km	0.38	cluster
11 Mar 02	NOAA	5.03 km	13.26 km	0.38	cluster
11 Mar 02	DMSP	3.57 km	10.88 km	0.33	cluster
11 Mar 02	MODIS	NA	NA	NA	cluster
13 Mar 02	NOAA	4.78 km	18.51 km	0.26	cluster
13 Mar 02	DMSP	4.50 km	23.04 km	0.20	cluster
13 Mar 02	MODIS	46.00 km	45.35 km	1.01	random

NA indicates data is not available.

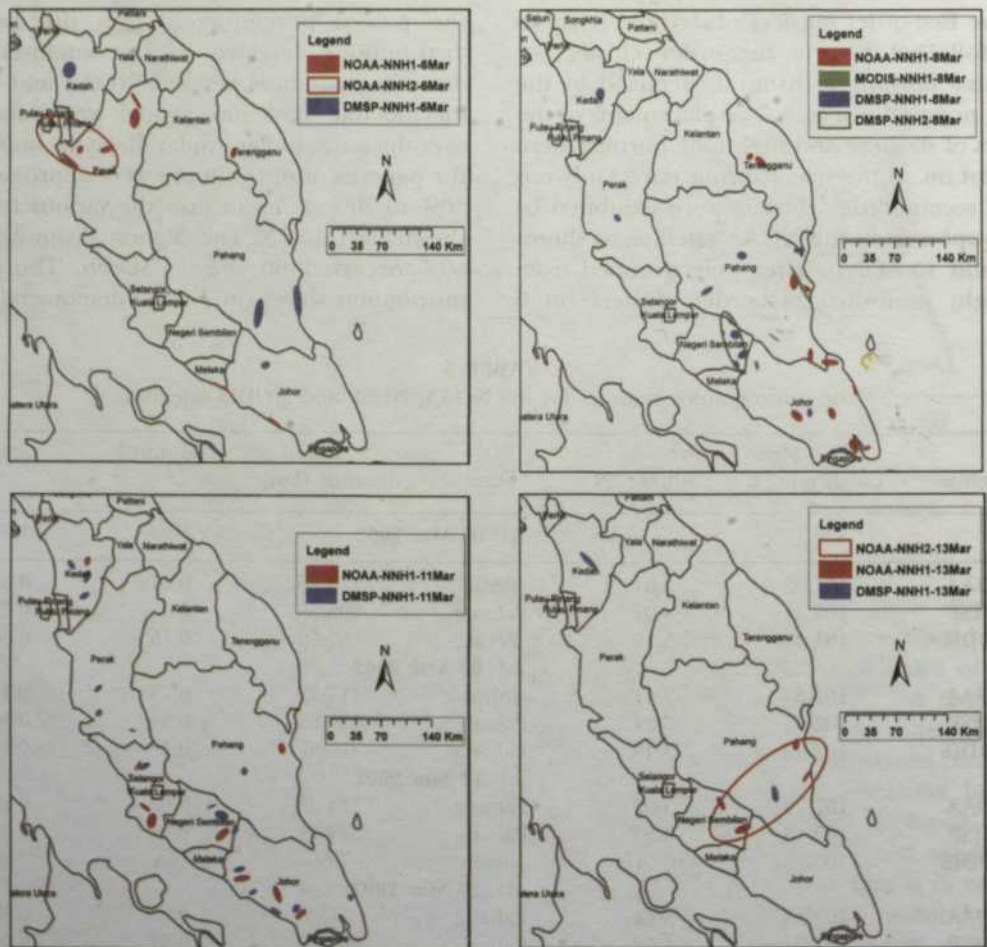


Fig. 5: The nearest neighbour hierarchical (NNH) cluster for selected days in March 2002 for the hotspots detected by the NOAA, DMSP and MODIS satellites. NNH1 represents the first-order cluster and NNH2 represents the second-order cluster

TABLE 5

The dissimilarity indices on selected days between NOAA and DMSP satellites

Dates	Dissimilarity index between NOAA and DMSP and the various states	
06 Mar 02	68.84	Johor, Kedah, Pahang, Terengganu
08 Mar 02	49.69	Kedah, Negeri Sembilan, Perak, Selangor, Perlis
11 Mar 02	32.05	Johor, Kedah, Melaka, Perak, Terengganu
13 Mar 02	47.75	Johor, Kedah, Melaka, Perak, Penang

most of the DMSP hotspots were clustered over the northwest and the southwestern coast of the peninsula, as specified by the mean centre location situated over the central region of the peninsula, with its larger standard distance, indicating the dispersion of its hotspots. In contrast, the NOAA hotspots were located over the northwestern states such as Kedah, Penang and Perak and substantiated by its mean centre and standard distance. Dissimilarity was found mainly in the states of Johor, Kedah, Pahang and Terengganu.

The distribution of hotspots on the 8th March with a high burning activity was illustrated by the concentration of hotspots over the northwestern, central and eastern coast in the states of Kedah, Kelantan and Pahang, but none over the southern or southwestern part of the peninsula. This shows that there exist a 50% difference in the patterns of hotspots between NOAA and DMSP particularly in the states of Kedah, Negeri Sembilan, Perak, Selangor and Perlis. Moderate dissimilarity indices indicating pattern change of hotspots between NOAA and DMSP on the 11th and 13th March mainly occurred over the states of Johor, Kedah, Melaka, and Perak.

One of the factors that hampered continuous monitoring over our equatorial region was the presence of clouds. The NOAA satellite only obtained partial coverage of the peninsula for only ten days within February and March. One of the disadvantages of the Terra MODIS satellite is that the overpass morning time is not coincident with the period of burning activities by the local farmers. MODIS does not always have a fixed area of daily coverage and its detection system requires a far higher saturation brightness compared to NOAA, which causes it to present a more conservative number of hotspots.

CONCLUSION AND RECOMMENDATION

The comparison of the hotspots over the Malaysian peninsula detected by the NOAA AVHRR, Terra MODIS and the DMSP OLS satellites showed that throughout February and March 2002, much biomass burning activities occurred during the drier and warmer than normal conditions over the peninsula. The different satellites with dissimilar overpass times, algorithms and resolutions showed different numbers of hotspots monitored throughout the day. The highest hotspots detected were by DMSP in February, followed by NOAA and the least by MODIS. Statistical relationships analysis for the hotspots from the three satellites was weak, with the strongest correlation between NOAA and DMSP.

There is a tendency of clustering in the burning patterns, particularly over the states of Perlis, Kedah and Pahang during early February in contrast to March. The day to day burning patterns on several case studies highlight a moderate index of dissimilarity, ranging from 30 % to 50% for most of the cases. Conspicuous vegetation fires were found over the paddy fields in the northwestern states of the peninsula in February compared to the logged over forests and peat land in Pahang during March.

In this study, the validation of hotspots with ground truth or sources of errors, resolution limitations or thresholds sensitivities of the hotspot detection from each satellite was not investigated, but raw data from the dataset of each satellite were utilized with the caveats and limitations. Although there may be a mismatch in accuracies, the study has successfully compared the spatial patterns of the hotspots derived from different satellites. The commotion of the active fires occurring throughout the day is revealed by the three satellites of differing overpass times. Burning activity that took place in the morning was found to be small, compared to the evening

deeds. Nighttime burning as detected by the DMSP satellite may also include the remnants from the evening activity, hence a higher correlation found between the locations of the hotspots detected by the DMSP and NOAA satellites.

In view of the above findings, conceivably an effort to synchronize data from all the satellites currently available and also future ones could be fostered so that a near-continuous 24 hour monitoring of the burning activities can be achieved where resources could be pooled and minimized for the benefit of end users within the Southeast Asian region. This is one of the goals of the Global Observation of Forest Cover - Global Observation of Land Dynamics (GOF-C-GOLD) under the Fire Implementation that may foster cooperation between the satellite producers and users (WWW5). Verification of the hotspots with ground truth data is therefore imperative to ascertain the accuracy of the hotspots, with improved algorithms.

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Preliminary Analysis of Quantitative Trait Loci Associated with Oil Quality in an Interspecific Cross of Oil Palm

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ABSTRACT

Amplified fragment length polymorphism (AFLP) and restriction fragment length polymorphism (RFLP) analysis are rapid and efficient techniques for detecting large numbers of DNA markers in oil palm. Both AFLP and RFLP markers were used to generate a genetic linkage map for oil palm. A map was constructed from AFLP and RFLP analysis of the progeny derived from an interspecific cross involving a Colombian *Elaeis oleifera* (UP1026) and a Nigerian *Elaeis guineensis* (T128). This interspecific cross was analysed to map genes associated with oil quality. For this cross, over 4,000 loci were detected using 53 AFLP primer pairs, and 60 informative cDNA probes as RFLP markers. Of these, 435 loci were informative and segregating in a 1:1 ratio, corresponding to DNA polymorphism heterozygous in one parent and null in the other. The results also showed that the male parent (Palm T128, a Nigerian *E. guineensis*) was more heterozygous than the female parent (Palm UP1026, Colombian *E. oleifera*) in the interspecific cross analysed. A framework map was generated for the male parent, T128, using JoinMap™ ver. 2.0. In the paternal *E. guineensis* map, 297 markers were ordered in 20 linkage groups (866 cM). The *E. guineensis* map was also used in scanning for quantitative trait loci (QTLs) controlling oil quality (measured in terms of carotene content and iodine value). QTLs associated with carotene content and iodine value (IV) were detected.

INTRODUCTION

The oil palm is a perennial crop which belongs to the genus *Elaeis* and the family Palmae. The crop was originally domesticated in Africa and is now extensively cultivated in Asia and Latin America as well. Within the genus *Elaeis*, two species are distinguished, which are, the economically important oil palm, *Elaeis guineensis*, native to Africa and a South American relative, *Elaeis oleifera*. Fortunately, the *E. guineensis* and *E. oleifera* hybridize readily, producing fertile offspring in spite of their difference in origin.

Oil palm is one of the most important oil bearing crops, being by far the highest oil yielder per unit of planting area. The past 30 years have

seen a rapid increase in the production of palm oil in the world, a greater than 7-fold increase from about 3 million tonnes in 1970 to over 23 million tonnes in 2001. Despite the progress, additional gains in agricultural productivity are needed at an ever faster pace due to competition from other vegetable oils and fats. Although traditional breeding continues to play an important role in yield enhancement, it is, however, impeded by the long selection cycle (10-12 years) (Obboh and Fakorede, 1989) and the enormous resources (land, labour and field management) required for oil palm breeding programmes. The ability to select early (at the nursery stage, perhaps) will thus have a great

impact in reducing the time and resources required for varietal improvement in oil palm. This makes marker-assisted selection (MAS) a very attractive proposition as it has the potential to reduce the time to develop new improved varieties.

The burgeoning field of molecular marker technology has provided tools for rapid gathering of genetic information about higher organisms, including agricultural species. In recent years, molecular markers have played a pivotal role in plant research, such as in studying evolutionary relationships among related species (Bennetzen and Freeling, 1993), the cloning of genes (Kazan *et al.*, 1993) and as diagnostic tools.

Perhaps the most widespread application of DNA markers has been in the construction of genetic maps, which can be used to determine the chromosomal locations of genes affecting either simple or complex traits (Paterson *et al.*, 1988). Genetic maps are useful in fundamental genetic research and tree improvement activities that include population management and marker-assisted breeding and selection. Molecular markers associated with selected traits of interest on a genetic map can be assayed in any tissue at any stage of development. This makes them ideal as selection markers in breeding programmes. The application of such DNA based diagnostics has been termed as molecular breeding (Leemans, 1993). The main advantage of this method over the conventional breeding process is its potential for reducing the time required for varietal development (Mazur and Tingey, 1995). The availability of probes of interest will allow breeders to select at the nursery stage thereby reducing the cost and time scale of breeding programmes.

Isozymes, restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) markers have been applied to oil palm to investigate genetic diversity (Shah *et al.*, 1994), to fingerprint clones (Mayes *et al.*, 1996; Cheah *et al.*, 1996) and in attempts at identifying markers for somaclonal variation (Cheah and Wooi, 1993; Rival *et al.*, 1998). A number of these marker systems have also been applied to genetic mapping in oil palm. RFLP had been applied to oil palm linkage mapping (Mayes *et al.*, 1997; Cheah *et al.*, 1999). The map reported by Mayes *et al.* (1997) harbors 97 RFLP markers in 24 groups of two or more markers and was generated by using the progeny of a

selfed *E. guineensis* cross. Recently, Moretzsohn *et al.* (2000) reported genetic linkage mapping for a single controlled cross of oil palm using RAPD markers and the pseudo-testcross mapping strategy. The status of genetic mapping in oil palm, however, is still considered preliminary at this stage. Furthermore, genetic linkage mapping of oil palm is currently carried out only in a limited number of laboratories. Considerable effort is required to generate dense maps and to resolve the number of linkage groups to 16, which is the haploid chromosome number of oil palm (Maria *et al.*, 1995). The ability to generate dense maps and to incorporate molecular marker technologies into existing breeding programmes can significantly accelerate many breeding and selection endeavors in oil palm.

In this study, the techniques of amplified fragment length polymorphism (AFLP) and restriction fragment length polymorphism (RFLP) were applied for construction of a genetic linkage map in oil palm. The map was constructed using progeny palms derived from an interspecific *E. oleifera* x *E. guineensis* cross of oil palm. The AFLP technique which was described by Vos *et al.* (1995) is known to be an efficient PCR based method for the identification of a large number of molecular markers. It has found widespread application in genetic mapping. The technique has been used to generate a large number of markers in the construction of linkage maps in potato (van Eck *et al.*, 1995) and rice (Nandi *et al.*, 1997). The technique is also useful for mapping quantitative traits (Powell *et al.*, 1997). Furthermore, AFLP markers linked to specific traits can be adapted to large scale, locus specific applications (Bradeen and Simon, 1998) and converted into diagnostic tools. In the oil palm, AFLP markers were found to be largely inherited in a Mendelian manner, indicating their suitability for the genetic mapping in this plant (Rajinder *et al.*, 1998).

Co-dominant RFLP markers are unequalled for many applications and have been successfully used in the construction of linkage maps for many plant species including oil palm (Mayes *et al.*, 1997; Cheah *et al.*, 1999). RFLP is often used to place complementary DNAs (cDNAs) on genetic linkage maps. The value of RFLP markers in this study lies in the fact that cDNA clones with known gene identities are used and these markers, being robust, will serve well as landmarks for the eventual integration of

available maps to form an oil palm consensus map. The main objective of this study was to construct dense genetic maps of the oil palm using both AFLP and RFLP markers for locating QTLs associated with oil quality.

MATERIALS AND METHODS

Plant Materials and Preparation of Genomic DNA

A mapping family of 77 F₁ palms derived from the cross UP1026 (*E. oleifera*) x T128 (*E. guineensis*) was analyzed. The palms were planted and evaluated at United Plantations, Teluk Intan, Perak. The female parent, UP1026, is a Colombian *E. oleifera* and the male parent, T128, is a Nigerian *E. guineensis* which produces oil with high iodine value (IV). DNA was prepared from young spear leaves by the method of Doyle and Doyle (1990).

AFLP Procedure

The AFLP assay was carried out by using the GIBCO BRL AFLP Analysis System 1 essentially as described in the manufacturer's manual, with some minor modifications. 350 ng of genomic DNA was digested with 3.2 µl of *EcoRI* and *MseI* (1.25 units/µl each) at 37°C for 4 hours. After heat inactivation of the enzymes at 70°C, the fragments were ligated to the *EcoRI* and *MseI* adapters in the presence of T4 DNA ligase at 20°C for 3 hours. A preselective amplification was then carried out by amplifying a 10-fold dilution of the ligation mixture.

For selective amplification, a selected *EcoRI* primer (with three selective nucleotides) was labeled with γ -³²P-ATP using T4 polynucleotide kinase. The labeled *EcoRI* primer was mixed with a selected *MseI* primer (three selective nucleotides containing dNTPs) at a ratio of 1:9 to form a primer master mix. The PCR reaction mixture contained 5 µl of a 30 fold diluted pre-amplified DNA, 5 µl of primer master mix, 0.5 unit of *Taq* DNA polymerase, 2 µl of a 10x PCR buffer in a final volume of 20 µl. PCR conditions as recommended by the manufacturer were adopted for use with the Perkin Elmer 9600 thermocycler.

Aliquots of the post PCR mixture were heated with an equal volume of formamide dye [98% (v/v)], 10 mM EDTA, 0.2% (w/v) bromophenol blue, 0.2% (w/v) xylene cyanol at 90°C for 3 min. A 5 µl sample was electrophoresed in a 6% (w/v) polyacrylamide sequencing gel with 7.5M urea. The gel was

dried and exposed to an X-ray film (Kodak XK-1) at -80°C for 2-3 days.

Southern Hybridization

DNA samples (20 µg) were digested with restriction enzymes as recommended by the manufacturer. Initially a sample of 10 palms was each digested with 14 restriction enzymes (*BamHI*, *BclI*, *BglII*, *DraI*, *EcoRI*, *HincII*, *HindIII*, *ScaI*, *SstI*, *XbaI*, *BstNI*, *HaeIII*, *RsaI* and *TaqI*). The restricted DNA fragments were separated by electrophoresis in 0.9% agarose gel in 1xTPE (90mM tris-phosphate buffer, 2mM EDTA pH 8.0) buffer and then transferred onto nylon membranes (Hybond N+, GE Healthcare) by vacuum blotting using 0.4M sodium hydroxide (NaOH) as the transfer buffer.

The set of 140 samples were then hybridized in turn with each candidate probe to identify the probe/restriction enzyme combination that gave a segregation profile. In the case of more than one enzyme showing polymorphism with a particular probe, the probe/enzyme combination that gave a single/low copy clear co-dominant profile was selected for screening the entire mapping family.

DNA probes were labeled with ³²P-dCTP (deoxycytidine 5'-[γ -³²P] triphosphate) by the method of Feinberg and Vogelstein (1984). *HindIII* and *HindIII/EcoRI* digested lambda phage DNA served as molecular weight markers for the estimation of the sizes of the hybridized fragments. Pre-hybridization and hybridization were carried out in glass tubes in a rotisserie oven at 65°C. Membranes were pre-hybridized for 3 hours in a pre-hybridization buffer as follows: 5 X SSPE solution (3M NaCl, 0.2M sodium phosphate, 20mM EDTA pH 8.0), 0.5% SDS, 5X Denhardt's solution (0.1% ficoll, 0.1% polyvinylpyrrolidone, 0.1% albumin bovine fraction V) and 100 µg/ml denatured herring sperm DNA. The pre-hybridization buffer was removed and replaced with hybridization buffer containing 5 X SSPE solution (3M NaCl, 0.2M sodium phosphate, 20mM EDTA pH 8.0), 0.5% SDS, and 100 µg/ml denatured herring sperm DNA. Labeled probes were denatured by heating in a boiling water bath for 10 minutes and plunging into ice before addition to hybridization buffer. The probe was added to a concentration of about 1-3 x 10⁶ cpm/ml. Hybridization was carried out overnight at 65°C. Hybridized membranes were washed twice in 2 X SSC (0.3M

NaCl, 30mM trisodium citrate pH 7.0) and 0.1% SDS at 65°C for 15 minutes each time, followed by a single wash in 1 X SSC (0.15M NaCl, 15mM trisodium citrate pH 7.0) and 0.1% SDS at 65°C for 10 minutes. The membranes were then autoradiographed at -80°C using X-ray films with intensifying screens for 7-10 days.

RFLP Probes

The RFLP probes used in this study were cDNA clones obtained from various cDNA libraries (young etiolated seedlings, mesocarp, kernel and root) constructed previously as described by Cheah (1996). cDNA clones from a subtracted flower library (Cheah and Rajinder, 1999) were also used to screen the mapping family.

The cDNA clones were picked at random from the various cDNA libraries. Plasmid DNA was prepared from individual clones by using the QIAGEN tip-20 plasmid prep kit (Qiagen, USA). The presence of the DNA insert was examined by restriction digestion (*EcoRI*) followed by electrophoresis on a 1.5% agarose gel. cDNA clones with insert sizes larger than 500 base-pairs (bp) were selected to screen for their abilities to detect RFLP in the mapping family. Probes for mapping were derived from the selected plasmids as polymerase chain reaction (PCR) amplified DNA fragments. Plasmids of selected probes were maintained as frozen glycerol stocks at -80°C.

Data Analysis

For AFLP and RFLP markers, segregation in the interspecific mapping family was scored on the basis of presence or absence of the band. The

parental origin of the markers was also recorded. Two separate data sets were obtained, one for each parent. The pseudo-testcross strategy (Grattapaglia and Sederoff, 1994) was used for analysis of the segregation data. In the pseudo-testcross configuration, each parent is, in turn, considered the heterozygous individual. Segregation is expected to be in the 1:1 ratio for bands present in the heterozygous parent (*Aa*) but absent in the homozygous parent (*aa*). A χ^2 test ($P < 0.05$) was performed to test the null hypothesis of 1:1 segregation on all the scored segregating bands. Table 1 illustrates the types of Mendelian segregation of the AFLP and RFLP patterns obtained in the offsprings of the interspecific cross scored as pseudo-testcross.

Map Construction

Map construction was carried out using the JoinMap™ version 2.0 computer programme (Stam and van Ooijen, 1995). The interspecific cross was analysed as a family resulting from a cross between two heterozygous diploid parents. The family type code "CP" was used in the analysis.

Linkage groups were identified by stepwise lowering of the LOD score from 7 to 2. LOD scores of 2 and 3 were found to give unlikely groupings. The LOD score of 4.0 was the lowest stringency at which acceptable linkages were performed. A ripple was performed after the addition of every three markers and map distances were calculated using the Kosambi map function. JoinMap™ constructs maps in three cycles. In the first two cycles, markers which exceeded the JUMP threshold are excluded. In the third cycle the markers excluded are inserted,

TABLE 1
Segregation of AFLP and RFLP markers in the progeny of the UP1026 (*E. oleifera*) x T128 (*E. guineensis*) interspecific cross

Parental genotypes	Expected Mendelian ratio	AFLP band pattern on autoradiogram		Remarks						
		Parents	Offspring	1	2	3	4	5	6	
Aa x Aa	3:1 (F ₂)	O	G	1	2	3	4	5	6	<i>E. oleifera</i> and <i>E. guineensis</i> parent heterozygous
		-	-	-	-	-	-	-	-	
Aa x aa	1:1 (testcross)	O	G	1	2	3	4	5	6	<i>E. oleifera</i> parent heterozygous
		-	-	-	-	-	-	-	-	
aa x Aa	1:1 (testcross)	O	G	1	2	3	4	5	6	<i>E. guineensis</i> parent heterozygous
		-	-	-	-	-	-	-	-	

with no restriction on the JUMP threshold. In this study, the ordering produced in the second cycle was used for map construction.

Quantitative Data Analysis

Oil was extracted from ripened bunches for use in subsequent analysis. The criteria used to determine ripened bunches were as described by Corley and Tinker (2003), one loose fruit per bunch (irrespective of palm height). A total of three bunches per palm were used for oil extraction and as such three independent readings of oil quality were determined per palm. The following traits were then evaluated: (i) carotene content and (ii) iodine value (IV). The carotene content and IV are quantitative traits and they represent measures of oil quality. QTL mapping analysis was performed using interval mapping implemented by MapQTL version 3.0 (van Ooijen and Maliepaard, 1996). Genomic wide significant threshold levels to declare a significant QTL was determined as described by van Ooijen (1999).

RESULTS AND DISCUSSION

Analysis of the E. oleifera (UP1026) x E. guineensis (T128) Mapping Family

(1) AFLP Analysis

The *E. guineensis* parent (Palm T128) and *E. oleifera* parent (Palm UP1026) and a sample of F₁ individuals were tested against a large number of combinations of *Eco*RI primers and *Mse*I

primers (both with three selective nucleotides). This was done in order to identify primer pairs which amplified bands that (i) were segregating in the mapping family and (ii) provided easy to read AFLP profiles. Each band was considered to represent a single locus of a dominant marker. On this basis, the presence of a fragment in a parent indicated that the parent was either homozygous dominant or heterozygous for that locus.

In the AFLP assay, a total of 26 AFLP primer pairs were used to screen the two parents and a small subset of the mapping family. The AFLP banding patterns obtained for one of these primer pairs is shown in Fig. 1. The number of bands observed in the population ranged from 13 to 99 (Table 2). A lower number of bands were usually obtained with primers which had CG dinucleotides. Primers with a high AT content usually gave higher numbers of bands. This indicates low frequency of CG dinucleotides in interspecific hybrids of oil palm, similar to that reported for other plant genomes (Moore *et al.*, 1993). Fig. 1 shows how the AFLP markers are easily scored as segregating alleles by the presence or absence of an amplified DNA band among the progeny. All 26 of the AFLP primer pairs analysed revealed scorable polymorphisms, thus illustrating the efficiency of AFLPs for analysis of the mapping family. Since all the AFLP primer pairs tested were suitable for detecting segregation in the interspecific cross, they were all used for screening the entire mapping family. The data obtained also provided confidence in

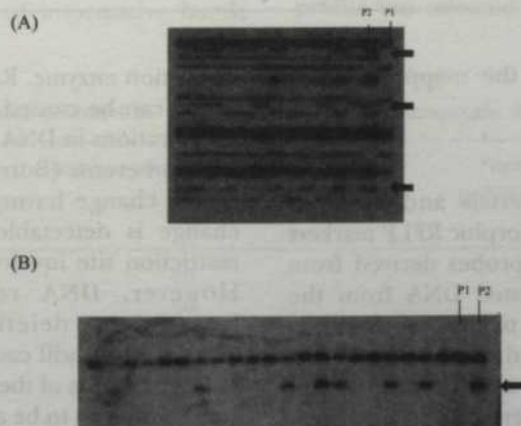


Fig. 1: Autoradiogram showing the segregating loci revealed by (A) AFLP markers and (B) RFLP markers. P1 is the female parent (*E. oleifera*), P2 is the male parent (*E. guineensis*) and the rest are the F₁ progeny. Arrows indicate segregating markers

TABLE 2
Segregation of AFLP markers in the pseudo-testcross revealed by a subset of 26 primer pairs used in analysis of the *E. oleifera* x *E. guineensis* progeny

Primer combination	Total no. of amplified bands	Segregation according to model		Total no. of segregating bands	No. of markers fitting goodness of fit to 1:1 ratio (P<0.05)
		Aa x aa (UP1026 heterozygous)	aa x Aa (T128 heterozygous)		
EACT/MCTA	57	-	11	11	9
EACT/MCAC	46	-	6	6	6
EACT/MCAA	99	-	11	11	8
EACT/MCTT	70	2	6	8	6
EACT/MCAT	75	2	11	13	9
EACT/MCTC	54	4	9	13	9
EACT/MCTG	45	-	4	4	4
EACT/MCAG	61	2	5	7	6
EACA/MCTC	52	1	7	8	7
EACA/MCAT	67	1	8	9	9
EACA/MCAA	86	4	8	13	10
EACC/MCTC	49	1	4	5	4
EACC/MCAC	34	-	2	2	1
EACC/MCAT	57	1	6	7	7
EACC/MCAA	67	1	9	11	8
EAAG/MCTG	87	-	12	12	10
EAAG/MCAG	54	-	8	8	6
EAAG/MCTC	78	2	4	6	4
EAAG/MCTA	78	5	15	20	11
EAAG/MCAC	70	-	8	8	8
EACG/MCAC	13	-	1	1	1
EACG/MCTC	34	-	1	1	1
EACG/MCAA	31	2	2	4	2
EAGG/MCAA	79	2	4	6	4
EAGG/MCAC	37	-	4	4	3
EAGG/MCAT	63	1	2	3	3
Total	1543	30	172	202	160
Mean	59.3	1.2	6.6	7.8	6.2

extending the analysis of the mapping family using RFLP markers.

(ii) RFLP Analysis

As described in the Materials and Methods section, screening for polymorphic RFLP markers was performed by testing probes derived from cDNA clones on the digested DNA from the parents and 10 individual palms. Parental and progeny DNA were digested with 14 restriction enzymes. A total of 288 cDNA probes from various cDNA libraries were tested for copy number and ability to detect segregation in the progeny. The results are tabulated in Table 3.

Of the 288 clones screened, 85, or 29.5%, showed polymorphisms with at least one

restriction enzyme. Restriction polymorphism in plants can be caused either by base substitution or alterations in DNA structure due to insertion/deletion events (Burr *et al.*, 1983). In the case of a base change having occurred at a locus, this change is detectable with the enzyme whose restriction site involves the base that is altered. However, DNA rearrangements such as insertions, deletions, inversions and translocations will cause the spatial arrangement of large regions of the genome containing several restriction sites to be altered. As such, this change can be detected with several enzymes. In this study, the majority of the probes used were also found to be polymorphic with more than one restriction enzyme, suggesting that polymorphisms

resulted more often from insertion/deletion events compared to individual nucleotide substitution.

(iii) Segregation Analyses of AFLP and RFLP Markers in the Mapping Family

The segregation of AFLP markers was examined with 76 F_1 plants derived from the cross of *E. oleifera* x *E. guineensis*. Although there are 77 F_1 plants from this cross, a previous study (Rajinder and Cheah, 1999) had shown that one of the progeny palms is a contaminant. This palm was thus excluded from the analyses.

The inheritance of the AFLP markers in the progeny was examined using the pseudo-testcross mapping strategy described by Grattapaglia and Sederoff (1994). In this strategy, it is assumed that in a cross between two individuals, markers which are heterozygous in one parent and null in the other will segregate in the ratio 1:1 in their F_1 progeny following a testcross configuration (Aa x aa or aa x Aa). The term "pseudo-testcross" is used because the testcross mating configuration of the markers is only inferred after analyzing for segregation of the markers in the offsprings. Either the male or the female parent can be fixed as the heterozygous individual contributing the segregating bands. The term "one-way" or "two-way" pseudo-testcross is used when one or both parents involved in the analysis are heterozygous, respectively.

A total of 3,792 AFLP loci were scored in the progeny using 53 AFLP primer pairs. 363 of these AFLP loci turned out to be informative and were segregating in the population progeny. On average, the number of informative bands

per primer pair was 7. The number of segregating bands obtained was also found to be significantly correlated with the total number of bands obtained ($r = 0.70$). This confirms our earlier results (Rajinder and Cheah, 1999) that an increase in pattern complexity of the total amplified DNA bands corresponds to a significant increase in informative markers for mapping.

Chi-square analysis was performed for each of the segregating bands scored to determine if segregation deviated from the expected 1:1 ratio. At a significance level of $P < 0.05$, 309 of these markers (about 85%) segregated in the expected ratio. Approximately 15% of the segregating bands displayed skewed segregation ratios. This shows that a high number of AFLP markers in the oil palm is stably inherited from parents to offsprings following the rules of Mendelian inheritance. Generally, all of the segregating markers scored were in the pseudo-testcross configuration, and either the male parent was heterozygous and the fragment was absent in the female parent (aa x Aa) or vice versa (Aa x aa). Surprisingly, the F_2 type of segregation pattern, where bands were common to both parents at the same locus and segregating in the 3:1 ratio in the progeny (Aa x Aa), were not detected.

Of the 85 RFLP probes showing polymorphisms with at least one restriction enzyme, 60 were selected for screening the entire mapping family. In cases where more than one restriction enzyme showed polymorphisms with a particular probe, the probe/restriction enzyme combination that showed clear, single copy profile was selected to screen the family.

TABLE 3
Polymorphisms detected by cDNA probes in the interspecific hybrid mapping family

cDNA library (source of probes)	Number of probes screened	Number of probes showing polymorphism with at least one restriction enzyme
Kernel	20	10
Mesocarp	40	15
Germinated seeds	60	20
Flower	120	25
Subtracted flower	20	5
Root	7	2
Others*	21	8
Total	288	85

* Probes obtained from research collaborators within/outside MPOB

The 60 probes screened detected 72 segregating RFLP loci (Table 4). All the RFLP loci were scored using the pseudo-testcross strategy as described above for AFLP markers. The male or female parent was fixed as the heterozygous individual contributing to the segregating band. At a significance level of $P < 0.05$, 68 of the 72 RFLP loci examined (94%), followed the expected 1:1 segregation ratio.

In this study, the majority of the segregating bands were found to be inherited from the male parent (palm T128, *E. guineensis*). Of the 435 (363 AFLP and 72 RFLP) segregating markers identified as segregating in the 1:1 ratio, 356 (290 AFLP and 66 RFLP) (82%) were heterozygous in the male parent and only 79 (18%) in the female parent, the Colombian *E. oleifera*. This confirmed that the male parent is more heterozygous than the female parent, *E. oleifera*. It is therefore concluded that it would be more appropriate to analyse this cross as a 'one-way pseudo-testcross' in which the male, *E. guineensis* is considered to be the heterozygous parent and the Colombian *E. oleifera* the homozygous parent.

As for the female parent, a different set of AFLP enzyme combination (e.g. *PstI/MseI*) or a different marker system (e.g. RAPD) may have to be used to scan different regions of the genome to generate sufficient markers which are informative for mapping. The low level of heterozygosity detected in the Colombian *E. oleifera* could be explained by the fact that *E. oleifera* is found in scattered areas in the South American country (Rajanaidu, 1986). This could have encouraged inbreeding, resulting in a relatively high homozygous genome.

(iv) Linkage Analysis

Linkage analysis was carried out using JoinMap™ version 2.0. As explained in the previous section, there were sufficient markers generated to develop a genetic map for the male *E. guineensis* parent, T128, while insufficient markers were available to generate a map for the *E. oleifera* female parent.

A total of 290 AFLP markers and 66 RFLP markers were used to generate a linkage map for the male T128 parent at a LOD score of 5.0. A detailed description of the genetic map is in progress with a graphical representation of the map. Essentially the genetic map consists of 20 linkage groups containing at least 3 markers

each. The total genetic distance covered by these markers was about 1,434 cM and the average distance between markers was about 5 cM. Generally, 297 of the markers analysed (about 83%) by the software could be linked to at least 2 other markers indicating good genome coverage. At a LOD score of 5.0, the RFLP and AFLP markers were found to be well distributed over all the 20 linkage groups.

The genetic map described above represent the relative order of genetic markers, and their relative distances from one another, along each chromosome of the oil palm. The oil palm has a haploid chromosome number of 16 (Maria *et al.*, 1995). As such more markers will definitely be required to resolve the map further to the basic chromosome number of 16.

(v) Quantitative Traits

A major objective in this study was to map QTLs associated with oil quality and yield in oil palm. The oil quality parameters analysed were carotene content and iodine value (IV). Oil that is high in carotene is desirable as there is a growing demand for natural sources of carotene for food colouring (Corley and Stratford, 1998). Iodine value, on the other hand, is a measure of the unsaturation of fats and oils. In order to allow for both edible and non-food usability of the oil, increasing the proportion of unsaturated fatty acids (particularly oleic acid) is desirable. Markers for these traits are important as they are potentially useful as early selection tools for oil palm breeding.

The two parents of the original cross could not be evaluated for these traits (carotene content and IV). Therefore, the trait values of the F_1 progeny cannot be compared to the parental generation. Interspecific hybrids typically display intermediate behaviour of these traits. However, since one of the individuals crossed is highly heterozygous, the F_1 is expected to be heterozygous, and a significant level of genetic variation is also expected to exist in the progeny. The existence of this genetic variation was explored in the QTL mapping experiment. Extreme phenotypes with trait values greater than and less than the standard deviation from the mean were observed for the carotene content and IV. The frequency distribution for the carotene content and IV did not differ significantly from normality assessed by Shapiro-Wilk statistics calculated by using PROC

UNIVARIATE (SAS, 1988). The mean, standard deviation and sample size for the carotene content and IV are shown in Figs. 2 and 3 respectively. Sample sizes for the analysis of oil quality were less than the total number of individuals genotyped. This is because some of the individuals of the cross have yet to bear fruits, and, as such, oil analysis could not be carried out. The correlation estimated among the two traits were, however, not significant at $\chi=0.05$ ($r = 0.42$).

(vi) QTL Analysis

QTL analysis was performed using interval mapping implemented by MapQTL version 3.0 (van Ooijen and Malieepard, 1996). In this study, for the interspecific cross, a linkage map was

generated for the male parent, T128, only. The linkage map for the T128 male parent was constructed with about 300 markers in 20 linkage groups. This marker density was considered suitable for use in QTL loci detection.

For carotene content, a total of two putative QTLs were detected in two different linkage groups (2 and 15). However, a genomic region was declared significant only when the genomic wide empirical threshold level calculated at the $P<0.05$ significance level was above 3.1. The threshold level was calculated according to van Ooijen (1999). Only the QTL detected at linkage group 2 met this criteria. Fig. 4 shows linkage group 2 with the significant QTL for carotene content. Generally, the genetic region controlling the QTL is located within the locus EAGG/

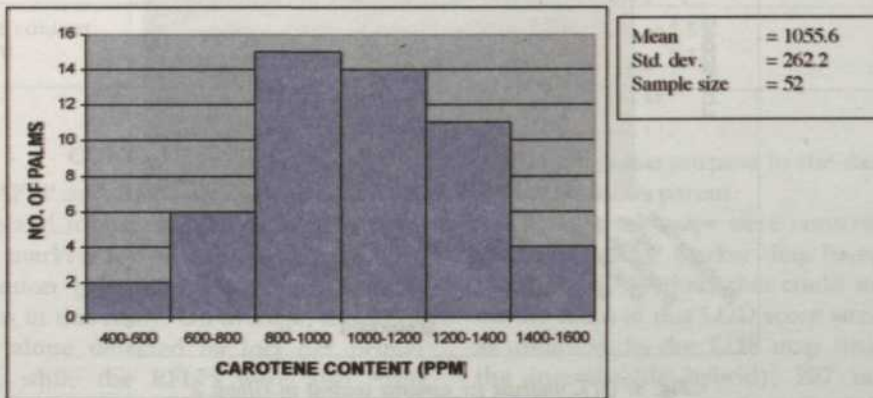


Fig. 2: Frequency distribution for carotene content in the interspecific hybrid F_1 progeny used for QTL mapping

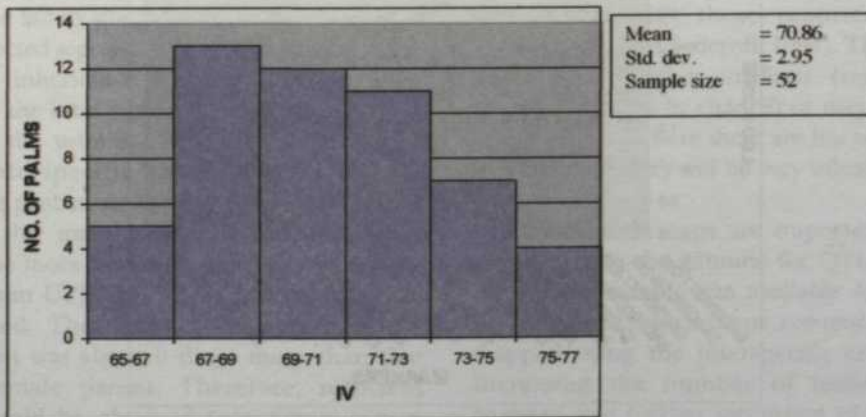


Fig. 3: Frequency distribution for IV in oil in the interspecific hybrid F_1 progeny used for QTL mapping

MCAA-340 and EACT/MCAT-590, in a region of about 20 cM. As for the IV parameter, a significant QTL was detected in linkage group 4 (Fig. 5). The region associated with the QTL was between the marker EACC/MCAT-195 and EAAG/MCAG-280, in a region spanning about 8 cM.

A summary of the QTLs detected in the analysis of the interspecific hybrid progeny is presented in Table 5. Generally the number of QTLs detected was low and this could be explained by the small size of the mapping family used. The cross has been redone and an additional 45 palms will be evaluated for both

map construction and QTL analysis in the future. Furthermore, in this study stringent genomic-wide empirical thresholds were used to declare a QTL. The stringent threshold is important to reduce the number of false positives (van Ooijen, 1999). Nevertheless the QTLs detected do explain a significant proportion of the variation observed for these traits (28% for carotene content, and 24% for IV). The markers as such hold promise as candidates for incorporation into the oil palm breeding programme to improve on oil quality traits.

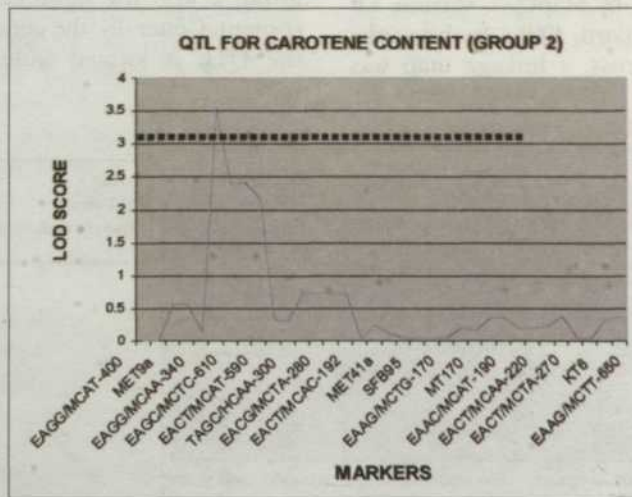


Fig. 4: QTL analysis for carotene content in Group 2

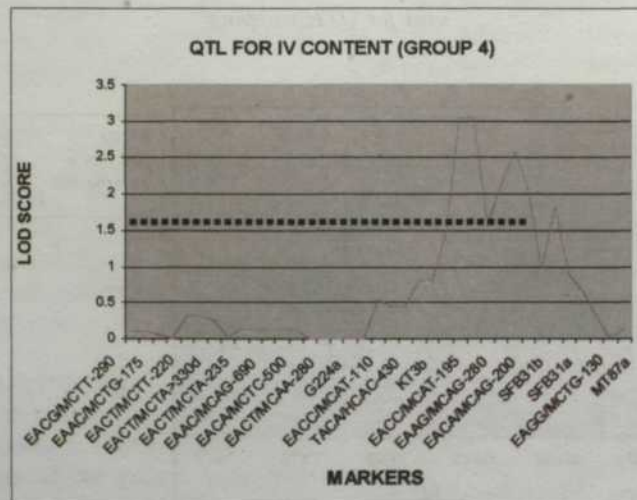


Fig. 5: QTL analysis for IV in Group 4

TABLE 4
Summary of RFLP and AFLP analyses of the interspecific hybrid mapping family

Type of markers	No. probes/primer pairs evaluated	No. of polymorphic loci identified	No. of markers showing 3:1 segregation	No. of markers showing 1:1 segregation in the gametes of		No. of markers meeting goodness of fit to 1:1 or 3:1 ratio
				T128	UP1026	
RFLP	60	72	-	66	6	68
AFLP	53	363	-	290	73	309

TABLE 5
QTLs for carotene content and IV found to be significant at an empirical significant threshold level of 3.10 ($P < 0.05$)

Trait	Linkage Group	Closest marker	LOD score	% variance explained by QTL
Carotene content	2	EAGG/MCAA-340	3.52	27.8
IV	4	EACC/MCAT-195	3.14	23.6

CONCLUSION

Both the AFLP and RFLP techniques proved to be reliable and robust techniques in generating molecular markers for oil palm. The high yield of information generated with these markers was obvious in this study. On average, the AFLP technique alone detected 59 loci per primer pair used, while the RFLPs were also easily detected in the mapping family analysed. The dominant AFLP markers and the co-dominant RFLP markers were also readily analysed in the interspecific hybrid progeny using the pseudo-testcross model. A majority of the markers (about 80%) in the family studied met the goodness of fit to the expected segregation ratios indicating stable Mendelian inheritance of the AFLP and RFLP markers in the interspecific hybrid cross analysed.

Using the two-way pseudo-testcross strategy in the interspecific cross, a data set for segregating probes was obtained for both parents. However, the male parent (Palm T128) was found to be more heterozygous than the female parent (Palm UP1026), as reflected in the data set collected. The data set generated for the male parent was about 6 times more than that for the female parent. Therefore, sufficient markers could be obtained to generate a map for the T128 male parent suitable for QTL detection. However, not enough markers were

available for this purpose in the data set for the UP1026 female parent.

Framework maps were constructed for the RFLP and AFLP marker data based on a LOD score of 5.0. Markers that could not be placed on the maps at this LOD score were considered as unlinked. In the T128 map (male parent of the interspecific hybrid), 297 markers were placed on a framework map defining about 1,434 cM of total map distance. There were 20 linkage groups with an average length of 72 cM.

The proportion of unlinked markers in the mapping population was about 20% and is comparable with those reported elsewhere (Grattapaglia and Sederoff, 1994). These unlinked markers are either artifacts (segregating in Mendelian ratios by chance) or they sample parts of the genome where there are few other markers, in which case they will be very valuable (Marques *et al.*, 1998).

Framework maps are important tools with which to scan the genome for QTLs. In spite of the limited sample size available for this study, QTLs controlling carotene content and IV were mapped using the interspecific cross progeny. Increasing the number of individual palms analysed and further saturation of the map will very likely lead to the discovery of more QTL positions controlling traits of interest. It is for

this purpose that efforts were made to re-create the interspecific cross to plant out sufficient numbers of the progeny. At present there are an additional 45 palms that have come to fruiting and will be included in future analysis.

The map produced in this study is specific to the individual used as parents. Such "single-tree" genetic maps therefore may not be representative of the species. For this reason, several maps need to be generated for a number of individuals in order to create a consensus map (Gentzbittel *et al.*, 1995). In the formation of a consensus map, single tree maps are linked using landmark markers, or anchor probes. Co-dominant markers such as RFLP especially serve well as anchor probes, since the probes are highly robust and stable. RFLP probes can also be easily shared by different laboratories and easily identified in different genetic maps, if the nomenclature for naming the probes is retained. It is for this reason that considerable efforts were made in developing and applying the RFLP technique in this study, although it was considerably laborious and expensive. This same concept extends to QTL mapping. The QTLs mapped in this study are individual specific. Homologies of linkage groups or homologies of QTLs in different oil palm maps have not yet been established at this time. Such homologies will have to await the localisation of common RFLP or other types of markers, for example simple sequence repeats (SSR) on the different oil palm genetic maps. The most important result to come from this study are that the QTL analysis has yielded a number of significant QTLs for oil quality, which influence a significant proportion of the total phenotypic variance (excess of 20%). To the knowledge of the authors, this is also the first report on the detection of QTLs associated with oil quality for oil palm. It is envisaged that the markers identified in this study at the very least can assist in the design of a more effective breeding programme for improving oil quality.

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Influence of Wet and Dry Seasons on the Breeding of Barn Owl and Its Relation to Rat Damage

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Keywords: *Tyto alba*, *Rattus argentiventer*, biological control, seasonal, crop damage

ABSTRACT

The effects of wet and dry season paddy crops on the breeding of barn owl (*Tyto alba*) and its relation to rat damage was studied in the district of Tanjung Karang, Selangor, Malaysia. The first site was at Sungai Burung where the nest box density was one box per 45 hectares of rice field. The second study site was in Sawah Sempadan which involved three experimental plots designed to investigate the effects of nest box density on rat damage, with densities of 1 box per 5, 10 and 20 hectares. The nest box occupancy rates for both seasons were 72.2% but can increase to 83.3% during the wet season crop. Mean clutch size during the dry and wet season crops were 5.38 and 4.07 respectively. Hatching success was 85.7% during the dry season and 79.2% during the wet season. Fledging success for both seasons were greater than 93%. Higher occupancy and hatching success during the dry season crop suggests that *T. alba* responded in a functional way in dealing with increasing rat numbers. Lower rates during the wet season crop serve to limit population growth of *T. alba*. Damage levels to paddy crop at Sungai Burung, during the tillering stage for both seasons were less than 2%. Damage levels during the booting and harvesting stages were also < 2% but increased to 3.22% and 3.39% respectively for the dry season crop. Higher crop damage level during the dry season can be associated with limited alternative food sources and the rats rely on paddy as their main food source. Damage levels at Sawah Sempadan were low throughout both crop seasons with mean crop damage levels of 0.65 + 0.10% for the 5 ha/box plot, 0.78 ± 0.28% for the 10 ha./box and 1.57 ± 0.15% for the 20 ha./box plot. This indicates that higher density of *T. alba* at Sawah Sempadan can control rats better throughout paddy crop seasons.

INTRODUCTION

Natural breeding of the barn owl (*Tyto alba*) for the purpose of rat control in rice fields in Malaysia was started in 1989 when the Malaysia Department of Agriculture established the first project in Tanjung Karang, Selangor, by providing artificial nest boxes (Shamsiah and Goh, 1991). *Tyto alba* tend to confine its feeding activities within a designated area around the nest box. By investigating the dietary remains, presented in the form of a pellet (Glue, 1974), the diet of *T. alba* can be determined, which also reflects the prey composition of the area (Webster, 1974).

A long-term study undertaken by Marti (1988) showed that food habits of *T. alba* shows a similar pattern in the same region, where it continuously preys on small mammals but rarely take other birds as food. Champbell *et al.* (1987) noted that in areas with similar climate and range of habitat, the prey species of *T. alba* remains consistent. They also noted that food habits of *T. alba* in British Columbia varied among different geographical areas, but *Microtus townsendii* remain the primary prey in all seasons, with the highest proportion recorded during the autumn season. In ricefield areas in Malaysia, *T. alba* mainly feeds on rats, especially the

ricefield rat *Rattus argentiventer* with occasional shrews and birds (Hafidzi and Naim, 2003).

Normally there are two rice planting seasons in a year in Malaysia. The first is the main season which coincides with the wetter months of the year and a second off-season which coincides with the dryer months. The objective of this study was to investigate the effect of wet and dry seasons on the breeding and feeding behaviour of barn owl.

MATERIALS AND METHODS

The study was conducted in Sungai Burung and Sawah Sempadan, Tanjung Karang Selangor Malaysia from January to December 2002. Rice is planted in these areas by direct seeding. There are two planting seasons per year: the dry season which lasts from April to August and, the wet season, from November to March. Rice varieties planted in the area are the MR 102, MR 129 and MR 185. In the Sungai Burung area, the time of planting is later than in Sawah Sempadan. For instance, in the dry season, planting in Sawah Sempadan started in February and in March for Sungai Burung. Eighteen artificial nest boxes set up by the Department of Agriculture in November, 2001 were chosen for this study. The nest boxes were modeled against the original design by Duckett (1976). The nest box density at Sungai Burung was 45 ha/box, although density may vary as these boxes were erected according to suitability of sites and ease of inspection. Nest occupancy rates were determined by the proportion of nest box with eggs, owlets and fledgling adults. Owlets were considered fledgling adults at 8 – 9 weeks from hatching (Smal, 1990). These parameters were used to compare the effects of wet and dry seasons on *T. alba* breeding.

Rat damage assessment was divided into three rice crop growth stages: tillering (4 weeks after seeding), booting (8-9 weeks after seeding) and harvesting (two weeks before harvesting) and divided into wet and dry seasons. The methods involved sampling along ten parallel linear rows of rice crop chosen at random in each plot. Ten quadrats of 0.25 X 0.25m for tillering stage and 0.5 X 0.5m, 5 meters apart were sampled along each row for 100 quadrats. The method of Buckle (1994) was used to assess rice crop damage.

$$\% \text{ Damage} = \frac{a \times c}{b + c}$$

Where : a = number of damaged hills out of 100 samples

b = number of undamaged tillers in the hills with damage

c = number of damaged tillers in the hills with damage

The rat damage levels at Sungai Burung were then compared with damage levels at Sawah Sempadan from three predetermined experimental plots with nest box densities of 5 ha, 10 ha and 20 ha/box. Fifteen artificial nest boxes were set up in August 30, 2001 in a designated area at Sawah Sempadan, where none has previously been established. The nearest nest box was more than one kilometer away to reduce impact on owl movement from neighbouring nest boxes. The boxes were arranged in three clusters consisting of five boxes covering an area of 5 (Plot A), 10 (Plot B) and 20 (Plot C) hectares as shown in Fig. 1. Analysis of variance (ANOVA) was used to statistically analyze damage levels between wet and dry seasons at each of the three planting stages; tillering, booting and harvesting. Correlation was used to compare nest box density and mean damage levels for both wet and dry seasons in combination.

RESULTS AND DISCUSSIONS

Breeding of Barn Owl in the Wet and Dry Seasons

The census showed there were two breeding seasons: during the wet season from January to April and during the dry season from June to September. Occupancy rates throughout the dry and wet seasons were consistent i.e. 72.2% (Fig. 2). However, November and December census showed that occupancy during the wet season can be as high as 83.3%. This indicates that occupancy rates may vary between planting season. Based on a yearly census carried out from 1993 – 1997, Hafidzi *et al.* (1999) showed that in the same area, the proportion of boxes with eggs were consistently higher during the first planting season (December to January) than the second planting season (July to August). They also found that the proportions of boxes with owlets were generally twice in February and

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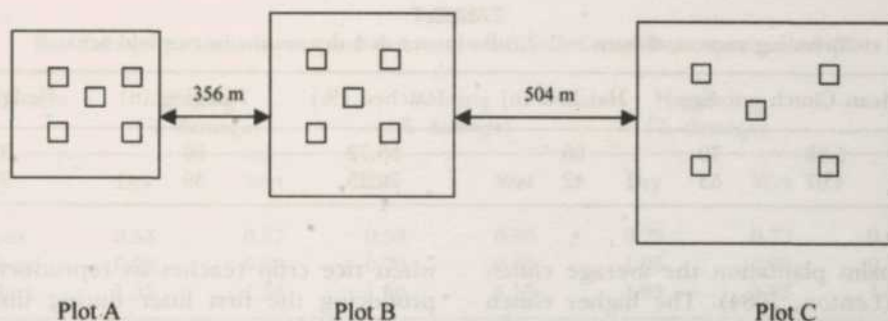


Fig. 1: Experimental plots representing three nest box densities. Plot A (5ha/box), Plot B (10 ha/box) and Plot C (20 ha/box). □ represents nest box

March compared to September. Therefore it can be deduced that wet and dry seasons may influence nest box occupancy rates, but these require a wider census over a number of years.

Smal (1988) showed that nest box occupancy of *T. alba* in an oil palm plantation varied from month to month. He also found there was a marked seasonal variation, with relatively few boxes being occupied in April and May and that peak occupancy, as high as 80 – 90%, were recorded from October to January (Smal, 1990). Lee and Ho (1999) found that nest occupancy rate in cocoa reached 70% at peak breeding season.

Table 1 shows that *T. alba* produce more eggs during the dry season i.e. mean clutch size of 5.38 compared to 4.07 during the wet season. Percent hatching was also higher in the dry season compared to wet season i.e. 85.7% and 79.2% respectively. This suggest that although there were indications that the proportion of nest boxes occupied were higher during the wet season, egg production per breeding pair may be higher during the dry season. This suggests

that *T. alba* may respond in a functional way towards prey availability (Erlinge *et al.*, 1984). In the dry season, food resources may be limited and therefore rats may rely heavily on the rice crop, increasing its density in the ricefield. This in turn may increase the hunting success of *T. alba*. Higher food intake leads to a higher clutch size and hatching success. However, the rate of fledging between dry and wet season was similar i.e. highs of 93%. This suggest that the lower clutch size and hatching success during the wet season is compensated for by correspondingly high fledging rates.

The high recruitment of *T. alba* in the preceeding dry season leads to a higher nest box occupancy in the wet season. Lower prey availability, as a greater range of food resource may lead to a dispersal of the rat population, which in turn cause lower egg production and hatching success. This compensatory mechanism leads to a stable *T. alba* population and keeps their numbers within the carrying capacity of the rice field habitat to prevent over predation on rats.

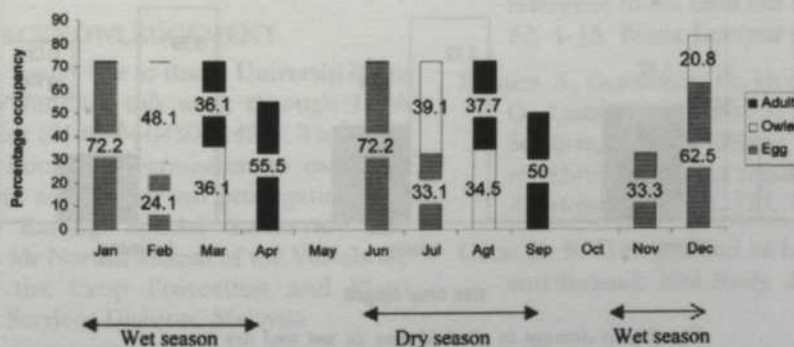


Fig. 2: Mean occupancy of barn owl *T. alba* in wet and dry season in ricefield area

TABLE 1
Breeding success of barn owl *T. alba* in wet and dry season in ricefield area

Season	Mean Clutch	Egg	Hatched (n)	Hatched (%)	Fledging (n)	Fledging (%)
Dry	5.38	70	60	85.72	56	93.3
Wet	4.07	53	42	79.25	39	93.8

In oil palm plantation the average clutch size is 6.6 (Lenton, 1984). The higher clutch size reflects the higher density of rats in oil palm plantations, which may reach 300 – 400 per hectare (Wood, 1969). In contrast, density estimates of rats in rice field ranged from 120 – 240 rats/ha. (Leung *et al.*, 1999). Oil palm plantations can sustain higher rat populations as food is almost available throughout the year, in the form of oil palm fruits, as opposed to paddy fields which are seasonal. The continuous breeding season from June/July to September/October and followed by a second clutch from October to January (Smal, 1990), supports this observation.

Rat Damage Analysis in the Wet and Dry Seasons

Incidence of rat damage in both wet and dry seasons at the tillering stage were less than 2% (Fig. 2). In all three development stages, damage to crop in the dry season were significantly higher than in the wet season (ANOVA; Tillering, $F = 4.41$, $P < 0.05$; Booting, $F = 4.56$, $P < 0.0001$; Harvesting, $F = 5.02$, $P < 0.001$). In the wet season, damage levels did not exceed 2% for all stages of growth. However, damage recorded in the dry season crop showed a marked increase from 1.34% to 3.22% and 3.39% at the booting and harvesting stages. Rats only start to breed

when rice crop reaches its reproductive stage, producing the first litter during the booting stage and subsequent litter during the ripening stage and shortly after harvest (Lam, 1983; Leung *et al.*, 1999). This partly explains the higher damage during ripening and harvesting. Also, during dry season, there is limited alternative food available. To maintain their sustenance, the rat population will converge on the paddy fields, leading to higher damage.

However, damage census in Sawah Sempadan showed that damage levels were low and stable for both the wet and dry season crops (Table 2). This can be attributed to the higher density of nest boxes in the area i.e. 20 ha, 10 ha and 5 ha per nest box. At such high density, *T. alba* has much better control of rats. Even, when comparing the three experimental plots in Sawah Sempadan, damage levels in the 20 ha. per nest box recorded a consistently higher damage levels than the other two plots i.e. 1.57 ± 0.15 % compared to 0.65 ± 0.10 % for the 5 ha/box plot and 0.78 ± 0.28 % for the 10 ha/box plot. Fig. 3 shows the extent of crop damage in Sawah Sempadan was highly correlated to nest box density ($R^2 = 0.96$).

Difference in % damage for all three crop stages between dry and wet seasons were significant.

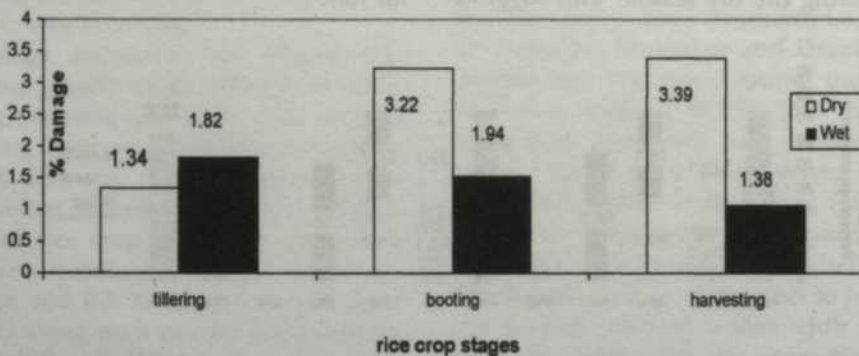


Fig. 2: Rat damage in ricefield area in wet and dry season

INFLUENCE OF WET AND DRY SEASONS ON THE BREEDING OF BARN OWL

TABLE 2
Rat damage patterns in wet and dry season at Sawah Sempadan experimental plots

Nest box density	Tillering (% damage)		Booting (% damage)		Harvesting (% damage)		Average
	Dry	Wet	Dry	Wet	Dry	Wet	
5 ha/box	0.53	0.57	0.58	0.66	0.79	0.77	0.65 + 0.10
10 ha/box	0.23	0.89	0.79	0.85	1.03	0.93	0.78 + 0.28
20 ha/box	1.57	1.54	1.36	1.53	1.83	1.62	1.57 + 0.15

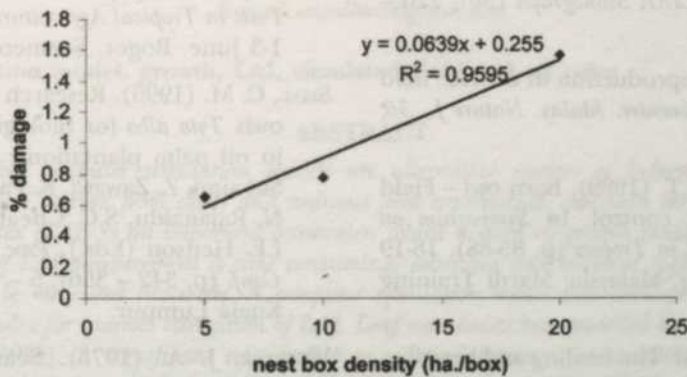


Fig. 3: Correlation between nest box density and % crop damage

CONCLUSION

Nest box occupancy by the barn owl, *Tyto alba*, varies between the dry and wet season crops, with possibly higher occupancy rates during the latter. Clutch size and hatching success is higher during the dry season crop but compensated for by the high fledging rates during the wet season crop. Damage levels were higher at the booting and tillering stages of paddy crop during the dry season. However, with high *T. alba* density, damage throughout crop stages in both seasons remain low and stable.

ACKNOWLEDGEMENT

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Leaf Area Index Model for Oil Palm FFB Yield Prediction

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Keywords: Simulation model, growth, LAI, simulated yield and oil palm

ABSTRACT

Accurate and reliable computer simulation models are alternative sources of information for replacing or enhancing information derived from costly and arduous field experiments, especially for perennial crops like oil palm. Leaf area index (LAI) is an important parameter, which is used in growth modelling of crops. Direct or destructive method of LAI measurement is time consuming, laborious and involves high cost. Hence, in the oil palm, LAI need to be measured indirectly. A computer simulation model was developed using Visual C++ to simulate leaf area index for indirect estimation of LAI. Leaf area index was modelled based on OPSIM approach (Van Kraalingen, 1985). A significant relationship was found between the simulated leaf area index and measured leaf area index. A good relationship was also found between simulated LAI and Yield of the oil palm, which could be used as an indirect means of yield prediction of the oil palm. In sensitivity analysis, results showed that the simulated LAI was sensitive to both Specific Leaf Area (SLA) and extinction coefficient 'k'. From this study, it can be concluded that the LAI could be fairly estimated using the computer program developed.

INTRODUCTION

Estimation of leaf area is one of the important variables to determine plant growth. Leaf area is a valuable index of plant growth and is related to the accumulation of dry matter, plant metabolism and yield. Crop quality and maturity may also be related to leaf area. Accurate estimates of leaf area index (LAI) are needed in ecosystem analysis because of the importance of canopy structure in gas, water, carbon and energy exchange (Chen and Cihlar, 1996; White *et al.*, 1997; Hu *et al.*, 2000). LAI is defined as the projected leaf area per unit of ground area (Lang *et al.*, 1991). This important parameter is difficult to measure directly in oil palm. Direct or destructive method is time consuming, laborious and involves high cost. It is often not permitted in oil palm for possible negative effects on yield. Hence, accurate, rapid and easiest approach for determination of leaf area in oil palm is essential.

Computer simulation modelling is increasingly being used to simulate LAI, forecast yields, determines risk, and/or provides support

for management decisions (McCown *et al.*, 1996; Heiniger *et al.*, 1997). Simulation models can replace expensive and time-consuming experiments and can be used as a research tool to support problem solving, risk assessment, and decision-making. It is especially convenient in perennial crops such as oil palm where field experiments are costly and time-consuming (Cox, 1996). The objective of this study was to develop a model to simulate leaf area index of the oil palm, which can be used to estimate crop yields.

LAI and Oil Palm Growth

Fig. 1 shows the basic growth process of the oil palm. Solar radiation is the major driving factor for physical growth and development of the plant. The physical yield of a crop can be determined by dry matter production, dry matter distribution and the dry matter content of the harvestable parts. Dry matter production is driven by physiological processes, such as gross photosynthesis, maintenance respiration and conversion of assimilate into biomass. In the short term, environmental conditions affect these

processes in different ways: light influences gross photosynthesis and temperature affects mostly maintenance respiration. Leaf area is an important determinant of light interception. Growth of the leaves is a function of the total dry matter production and the fraction of dry matter partitioned into the leaves. The growth of the oil palm can be divided into vegetative growth and generative or reproductive growth. In other words, total dry matter consists of vegetative dry matter and bunch dry matter. Dry matter production is determined as the sum of production in fronds, bunches and trunk. Bunch dry matter production is usually estimated from fresh bunch weight and a factor of 0.53 is used when converting fresh to dry weight (Corley, *et al.*, 1971).

Simulation of Leaf Area Index

Leaf area index is the key parameter of a plant growth model, which is related to photosynthesis, accumulation of dry matter, plant metabolism and yield. Therefore, simulation of LAI is important to simulate yield. LAI can be simulated in two ways, such as based on growth function of the leaflet and experimental empirical formula.

In this model, LAI was simulated based on growth function and then adjusted based on experimental findings.

Leaf area index is a function of leaf area per palm where the Leaf Area per Palm (LAP) can be expressed as a function of the leaflet weight and specific leaf area as shown in equation 1:

$$LAP = f(SLA, WOL) \tag{1}$$

where, SLA is the specific leaf area in m²
WOL is the weight of leaflet in g

SLA or specific leaf area is dependent on age and varies from 3.5 – 8.0 m²/kg (Corley, 1971). However, based on experimental results, SLA was assumed to vary from 2.5 - 5.5 m²/kg in this model.

Mathematically, leaf area index (LAI) can be calculated as shown in equation 2:

$$LAI = LAP \times PD / 10000 \tag{2}$$

where, PD is the planting density in number of palms per hectare.

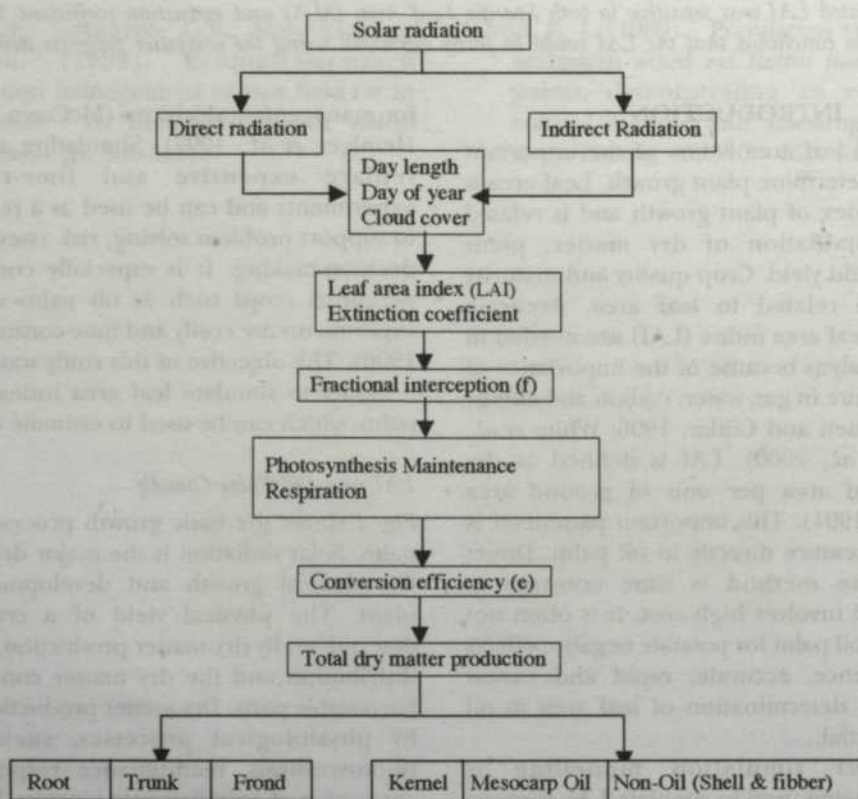


Fig. 1: Basic growth process of the oil palm (Modified from Weng, 1999)

The following shape factor and Dry-mass models were used to adjust the simulated LAI. The Dry-mass model is as shown in equation 3,

$$L_a = 99 \times L_m \quad (3)$$

where, L_m is the mass of leaflet in g, and L_a is the rectangular leaflet area in cm^2 . The Shape factor model is represented in equation 4:

$$L_{ac} = 0.80 \times (L \times W) = 0.80 \times L_a \quad (4)$$

where, L_{ac} is the actual leaflet area in cm^2 , L is the leaflet length in cm, and W is the leaflet width in cm. So, actual leaflet area can be calculated as shown in equation 5:

$$L_{ac} = 99 \times 0.80 \times L_m \quad (5)$$

Fig. 2 shows the flow charts of oil palm LAI simulation model (OPLAIM). In the algorithm, as represented in the flow chart, leaves growth (increasing) rate, death (decreasing) rate due to senescence and pruning, and weight of leaves were calculated. Then leaf area per palm and

leaf area index were calculated. Direct (destructive) method was used to measure LAI from field (Awal, 2006).

RESULTS AND DISCUSSION

Relationship between Simulated LAI and Palm Age

Fig. 3 shows the relationship between simulated LAI and the palm age. A significant linear relationship was found between palm age and simulated LAI. Results indicate a high degree of association ($r = 0.97$) between palm age and simulated LAI with a standard error of estimation of 0.9. Simulated results also show that LAI of palms increases up to year 16 and then remains constant beyond that age.

Relationship between Simulated LAI and Measured LAI

Fig. 4 shows the relationship between simulated and measured LAI. The simulated LAI was overestimated compared to the measured LAI. Results show that the simulated LAI was overestimated by 13% for younger (2 to 3-year old) palms and by about 40% for palms over 15-years old.

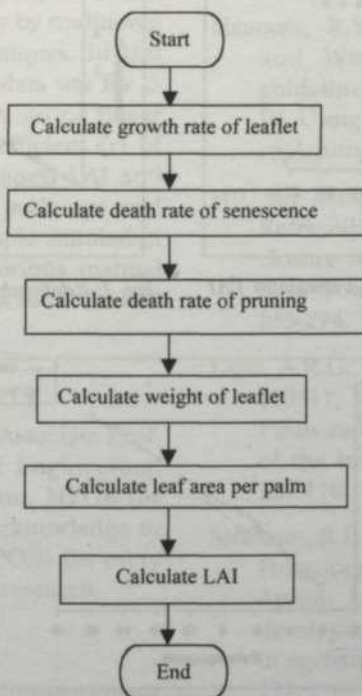


Fig. 2: Flow chart of the oil palm LAI simulation model

Palm Age versus. Simulated LAI and Measured LAI

Fig. 5 shows the simulated and measured LAI for 2 to 16-year old palms. The simulated LAI values were approximately the same as the actual LAI values for immature palms. However, the simulated LAI values were higher compared to measured LAI values for older palms. This is not surprising, since the simulation was done based on ideal conditions, whereas field data was collected from different treatment plots. The simulated LAI can be considered sufficiently accurate for comparison with the measured LAI.

Relationship between Simulated LAI and Simulated Yield

Fig. 6 shows the relationship between simulated LAI and simulated yield. A strong linear relationship was observed between the simulated LAI and the simulated yield with correlation coefficient, $(r) = 0.96$ and coefficient of determination $(R^2) = 0.92$ with the standard

error of estimation being 1.21. Results indicate that the simulated yield can be adequately estimated from simulated LAI with $P \leq 0.001$.

Sensitivity Analysis

Sensitivity analysis was used to ascertain how a given model output varies with the input parameters. On the basis of this experience, specific leaf area and radiation extinction coefficient (k) were used to determine this sensitivity on the simulated LAI (Fig. 7). Results show that LAI was highly sensitive to both parameters. Fig. 7 shows that the sensitivity of LAI to both parameters was similar in case of increasing values of these parameters, however, specific leaf area (SLA) was more sensitive than "k" in case of decreasing values of these parameters. It is therefore important to accurately estimate the value of "k" to be used in the model in order to minimize errors in the estimation of LAI of oil palm.

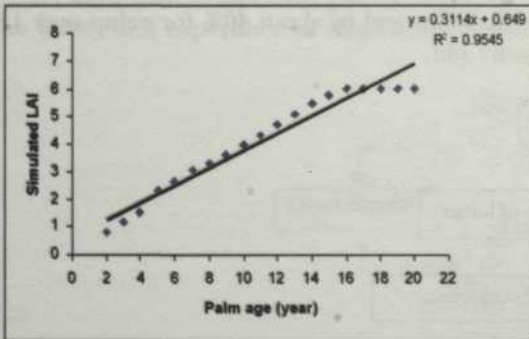


Fig. 3: Relationship between palm age and simulated LAI

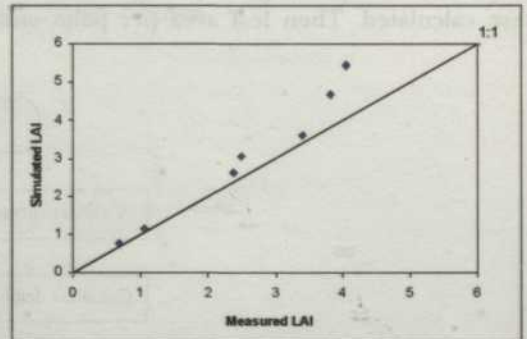


Fig. 4: Relationship between measured LAI and simulated LAI

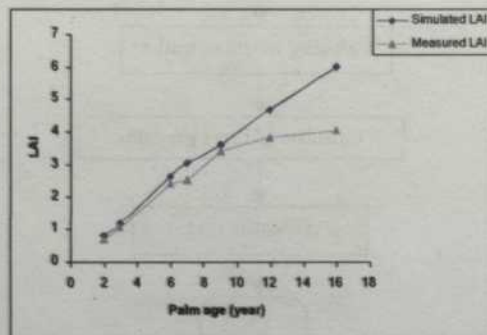


Fig. 5: Simulated LAI and measured LAI for 2 to 16-year old palms

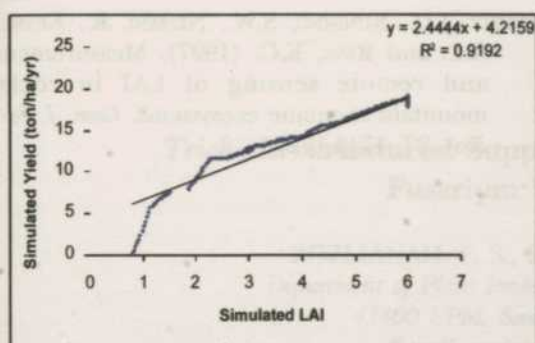


Fig. 6: Relationship between simulated LAI and simulated yield

CONCLUSION

This leaf area index (LAI) model enables researchers or farm managers to evaluate palm management strategies. It can trigger palm and soil management strategies if they are used as warning systems, e.g. monitoring leaf growth in relation to drought risks and nutrients deficiency. The LAI simulation results were reasonably similar to the field data. However, the predictions by simulation models may differ from actual field observations for a variety of reasons, and such deviations can be revealed instantly by traditional or by new field monitoring techniques. In this study, the LAI field experimental data was for 2-16 year old palms (Awal, 2006). A strong linear relationship with a correlation coefficient (r) of 0.96 was found between the measured LAI and the simulated LAI, and between palm age and simulated LAI ($r = 0.97$). This simple simulation technique could replace the laborious manual method or costly instruments, to predict the LAI and oil palm FFB yield.

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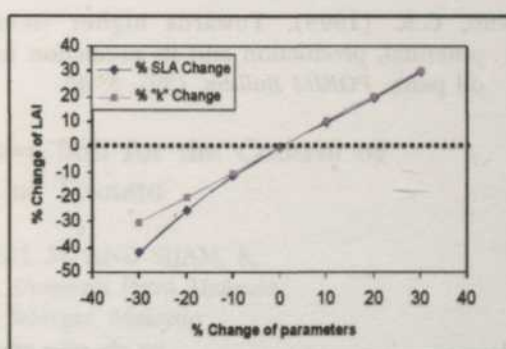


Fig. 7: Sensitivity of simulated LAI with change of SLA and extinction coefficient

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Trichoderma-induced Suppressive Soil for the Control of Fusarium Wilt of Tomato

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Keywords: *Fusarium oxysporum* f. sp. *lycopersici*, disease progress, tomato, *Trichoderma*-induced suppressive soil

ABSTRACT

A study was carried out to evaluate the efficacy of *Trichoderma*-induced suppressive soil on growth of tomato cv Baccarat 322 and on wilt disease caused by *Fusarium oxysporum* f. sp. *lycopersici* (Fol). Dry preparation of *T. virens* (UPM 23), *T. harzianum* (UPM 40), singly and as mixtures (UPM 2340) with organic compost as carrier were amended into soil mixture as treatments to induce disease suppressiveness. The wilt fungus at the inoculum level of 100 mL plant⁻¹ (9×10^6 spore mL⁻¹) caused significant suppression in growth and yield. However, the *Trichoderma*-induced suppressive soil checked the suppressive effect of the fungus leading to significant increase in root dry weight and yield compared with the inoculated control. Disease incidence expressed as the area under the disease progress curve (AUDPC) was highest for the control plants and lowest for plants treated with UPM 2340 followed by UPM 40 and UPM 23. The disease progress rate was significantly lower in plants treated with UPM 2340 ($r_m = 0.01$) compared to non-treated plants ($r_m = 0.75$). Rhizosphere population of the introduced *Trichoderma* (colony forming units g⁻¹ soil) gradually decreased with an increase in frequency recovered from the tomato roots, suggesting an ability of the *Trichoderma* to colonize the roots.

INTRODUCTION

The production of tomato (*Lycopersicon esculentum*, Mill.) in Malaysia, confined mainly to the highlands because of the mild temperature, is threatened by the wide spread of vascular wilt, a disease commonly associated with *Fusarium oxysporum* f. sp. *lycopersici* (Fol). Control measures such as the use of resistant varieties and chemicals have been found to be erratic or not long lasting. This could be due to the unavailability of the acceptable resistant cultivars and, moreover, Methyl Bromide Chloropicrin (MBC), a fumigant commonly used for the control of *Fusarium* wilt has been deregistered due to the implications for soil and water pollution.

The success of biological control through manipulation of antagonistic microorganisms such as of *Trichoderma* species, has been extensively studied in field and glasshouse crops. Introduction of *T. harzianum* (UPM 40) and *T. virens* (UPM 23) applied as granules or as dry

powder has significantly promoted plant growth in several crops, with significant increase in the development of the root system (Ismail, 2001; Franklin, 2002). They have also been shown to suppress certain root diseases of vegetable crops (Jinantana and Sariah, 1998; Ibrahim, 2005). These antagonistic fungi can proliferate in the rhizosphere colonizing the roots, creating an environment unfavourable for the growth and sporulation of the pathogen. Henis *et al.* (1979) observed the development of suppressiveness after four to five successive plantings of radish, but observed no correlation between onset of suppressiveness and the *in vitro* antagonism of *Rhizoctonia solani* by the resident soil microflora. However, suppressive soils possessed higher populations of *Trichoderma* spp. than the corresponding conducive soil. This study was carried out with the aim of evaluating the effect of *Trichoderma*-induced suppressive soil for the control of *Fusarium* wilt of tomato.

MATERIALS AND METHODS

Preparation of Trichoderma Inoculants

Stock cultures of *Trichoderma virens* (UPM 23) and *T. harzianum* (UPM 40) were obtained from the Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia. UPM 23 and UPM 40 were cultured on potato dextrose agar (PDA) and incubated at room temperature ($28 \pm 2^\circ\text{C}$) for one week.

Molasses and rice flour were used as culture substrates for the mass production of the *Trichoderma* inoculants as it has been shown to produce abundant conidia and mycelial biomass (Ibrahim, 2005). Compost prepared from oil palm trunk and chicken dung (OPTCD) was used in this study as a carrier for the *Trichoderma* inoculants and/or to provide a ready food source for the initial establishment of the inoculants. The physical and microbiological properties of the compost were as determined (Ibrahim, 2005). The freeze-dried biomass of *Trichoderma* (UPM 23 and UPM 40) and the mixture of UPM 23:40 (UPM 23: UPM 40; 1:1 w/w) were incorporated into the carrier (OPTCD) in the ratio 1:10 w/w fungal biomass: OPTCD. The initial colony forming units (cfu) of the formulated mixtures were determined and expressed as g^{-1} dry substrate.

Preparation of Trichoderma-Induced Suppressive Soil

Potting medium was prepared by mixing non sterilised soil mixture (3:2:1 v/v, mixture of top soil, sand and peat) with the respective *Trichoderma* preparations at 0.5% (w/w), respectively to induce 'suppressive soil'. Three kilograms of each mixture were placed in pots and allowed to incubate for four days at field capacity to allow for the proliferation and establishment of the *Trichoderma* inoculants in the soil. The populations of *Trichoderma* inoculants in the soil for each treatment were evaluated at the end of the experiment.

Effects of Trichoderma-induced Suppressive Soil on Plant Growth and Disease Suppression

Individual tomato seedlings cv Baccarat (4-6 leaves stage) were transferred into individual pots containing the *Trichoderma*-induced suppressive soil and allowed to establish for two weeks at field capacity. The seedlings were then inoculated with 100 mL Fol inoculant with a spore count of 9×10^6 conidia/mL. Treatments carried out include plants treated with compost

alone (T1); UPM 23 (T2); UPM 40 (T3); mixture of UPM23 and UPM 40 (T4) and control (T4, sterilised distilled water) both for the infested and non-infested soil. The factorial experiment was conducted in a glasshouse with pots arranged in completely randomized design (CRD) replicated four times with three seedlings per replicate. Data were recorded as mean for each replicate. All seedlings were watered daily and fertilized fortnightly with NPK Blue (12:12:17) at a rate of 3 g seedling⁻¹.

Beneficial effects of *Trichoderma*-induced suppressive soil on plant vigor were assessed based on root mass and yield. Total yield of marketable fruits were harvested 12 weeks after transplanting and weighed. At the end of the experiment, three plants from each replicate were uprooted and shaken vigorously to remove all adhering potting medium. The roots were separated and placed in the oven at 65°C for 48 hours for determinations of dry weight.

Disease incidence (DI) was calculated based on the foliar-associated symptoms, according to the formula (modified from Campbell and Madden, 1990). Plants were considered infected when they expressed symptoms of epinasty, yellowing of lower leaves, wilting or marginal necrosis of the remaining leaves. The Area Under Disease Progress Curve (AUDPC) was then assessed using the same data plotted as disease progress curve based on the formula: $\text{AUDPC} = \sum_{i=1}^{n-1} (y_{(i+1)} + y_i / 2) (t_{(i+1)} - t_i)$, where n = the number of assessment time; y = disease incidence and t = time (weeks). The disease progress rate expressed as the slopes of the disease progress curve was obtained by the multiple regression analysis using the Sigma Plot Software Program (SPS Version 9).

Proliferation and Establishment of Trichoderma in the Rhizosphere and on Roots of Tomato

Trichoderma population in the rhizosphere and roots of tomato was determined following the soil dilution plate technique on *Trichoderma* selective medium (TME) (Papavizas, 1981), and expressed as colony forming units g^{-1} soil (cfu g^{-1}). Roots of tomato were sampled according to Parke (1991) to determine the colonizing ability of the introduced *Trichoderma* inoculants and were expressed as cfu g^{-1} roots.

All data were arc-sine transformed and subjected to ANOVA using Statistical Analysis System (SAS) computer program. Results

showing significant differences were then subjected to mean comparison using Tukey's Studentized Range Test ($HSD_{0.05}$).

RESULTS

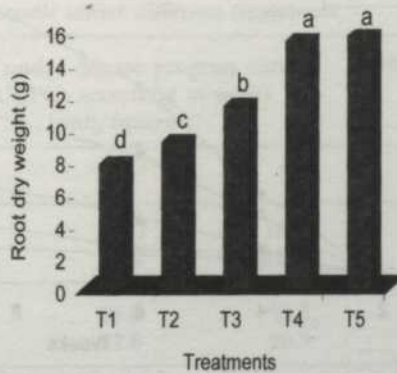
Effects of Trichoderma-induced Suppressive Soil on Plant Growth and Disease Suppression

Trichoderma-fortified compost incorporated into potting medium to induce soil suppression to Fusarium wilt had a significant effect on the vegetative growth of tomato plants based on root dry weight and yield. In the absence of *F. oxysporum f. sp. lycopersici*, plants grown in the Trichoderma-induced suppressive soil gave significantly higher values of root dry weight compared to the control (Fig. 1A). Fusarium infection had a detrimental effect on root dry weight of tomato plants (Fig. 1B). However, in

the presence of *Trichoderma*-induced suppressive soil, the tolerance of tomato to Fusarium infection increased and this promoted vigor through an increase in root dry weight of the infected plants. Results showed that UPM 2340 and UPM 40 gave the highest effect with mean values of 14.41 g and 14.08 g, respectively. The control plants produced 2.83 g of root dry weight which was significantly lower than UPM 23 (9.85 g) and compost alone (5.09 g).

Tomato plants grown in the *Trichoderma*-induced suppressive soil showed a significant increase in total fresh weight of fruits. The total fresh weight of fruits was 512.08 g, 511.62 g, 454.97 g and 382.76 g for UPM 2340, UPM 40, UPM 23 and compost alone (Table 1), respectively for the non infested soil. The control plants produced only 306.03 g. The wilt

(A)



(B)

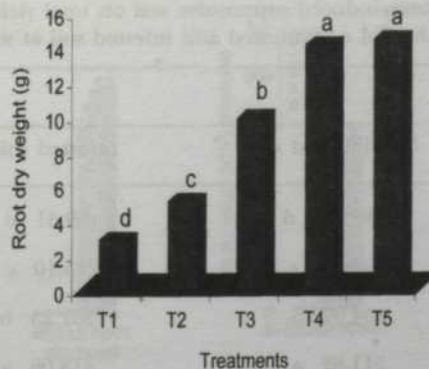


Fig. 1: Effect of Trichoderma-induced suppressive soil on root dry weight of tomato plants in the *Fol* non-infested (A) and infested soil (B) at 12 weeks after treatment. Bars with different letters are significantly different using $HSD_{0.05}$. T1: Control (Sterilised distilled H_2O), T2: Compost alone, T3: UPM 23, T4: UPM 40, T5: UPM 2340

fungus at inoculum level of 100 mL plant⁻¹ (9×10^6 spore mL⁻¹) caused significant suppression in yield. However, the *Trichoderma*-induced suppressive soil checked the suppressive effect of the fungus leading to significant increase in root dry weight and yield compared to the inoculated control. The reduction in total fresh weight of fruits was highest with value of 64.28% (T1), followed by 25.51% (T2) as compared to 18.08%, 18.28% and 20.60% for T5, T4, and T3, respectively (Table 1).

Disease incidence was highest in control plants (T1) and lowest in plants treated with UPM 2340 (T5) followed by UPM 40 (T4), UPM 23 (T3) and compost alone (T2) (Fig. 2).

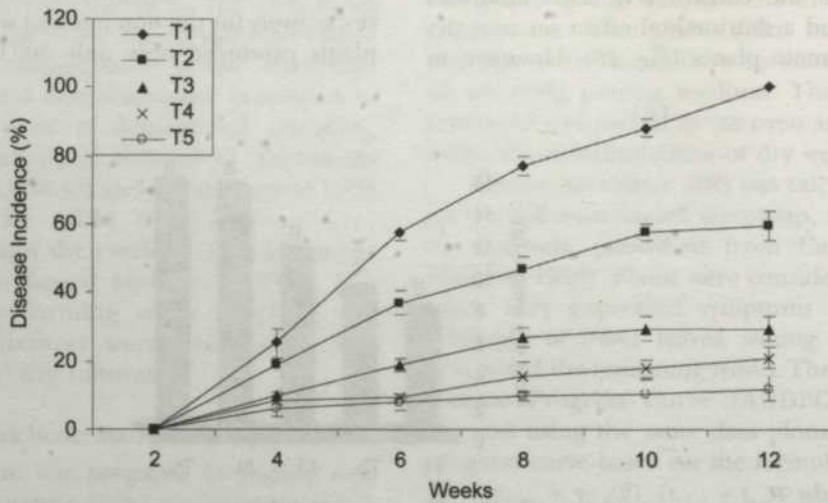


Fig. 2: Effect of different treatments (T1-T5) on the development of *Fusarium* wilt on tomato. T1: Control (Sterilised distilled H₂O), T2: Compost alone, T3: UPM 23, T4: UPM 40, T5: UPM 2340. Bars represented the standard error

TABLE 1
Effect of *Trichoderma*-induced suppressive soil on total yield of fruits per plant in the Fol non-infested and infested soil at week 12.

Treatment	Total fresh weight of fruits (g)		
	Non-infested soil	Infested soil	Reduction (%)
T1	306.03 d	109.31 d	64.28a
T2	382.76 c	285.10 c	25.51b
T3	454.97 b	361.23 b	20.60c
T4	511.62 a	418.08 a	18.28 c
T5	512.08 a	419.52 a	18.08c

Means with different letters within a column are significantly different using HSD_{0.05}. T1: Control (Sterilised distilled H₂O), T2: Compost alone, T3: UPM 23, T4: UPM 40, T5: UPM 2340.

as treatments T3, T4, and T5. This could possibly be due to the population of indigenous *Trichoderma* present in the compost and the presence of high saprophytic fungi that can cause strong competition for nutrients and space, as the soil mixture used in this study was not sterilized. In addition to disease incidence, the progress in disease development was also evaluated based on the area under the disease progress curve (AUDPC). The symptoms development was delayed in the *Trichoderma* treated plants as seen by the Area under Disease Progress Curve (AUDPC) values at week four until week 12 compared to control (Table 2), with the disease progress rate (slope) highest for T1, followed by T2, T3, T4 and finally T5

($r_m = 0.75$, $r_m = 0.09$, $r_m = 0.04$, $r_m = 0.02$ and $r_m = 0.01$, respectively).

Proliferation and Establishment of Trichoderma in the Rhizosphere and on Roots of Tomato

Proliferation and survival of *Trichoderma* spp. was assessed at week 12 from both the rhizosphere of Fol non-infested and infested soil mixture and also the roots of tomato. The populations were higher on roots than rhizosphere for both main plots of Fol non-infested and infested soil (Fig. 3). The results showed that populations of *Trichoderma* in the rhizosphere were significantly lower than on roots due to the movement of *Trichoderma* populations from potting medium towards the roots.

TABLE 2
Area Under Disease Progress Curve (AUDPC) and disease progress rate of Fusarium wilt under different treatments

Treatment	² Area under disease progress curve (AUDPC) according to weeks (unit/square ²)					Disease progress rate (slope) at week 12 (unit/week)
	4 ¹	6	8	10	12	
T1	25.3	83.1	134.7	164.9	188.0	0.75
T2	19.1	56.1	84.0	104.8	117.3	0.09
T3	10.0	28.9	45.8	56.4	59.0	0.04
T4	8.5	18.0	25.0	33.3	38.8	0.02
T5	6.0	13.8	17.6	20.3	22.5	0.01

¹ weeks ² AUDPC values

T1: Control (Sterilised distilled H₂O), T2: Compost alone, T3: UPM 23, T4: UPM 40, T5: UPM 2340

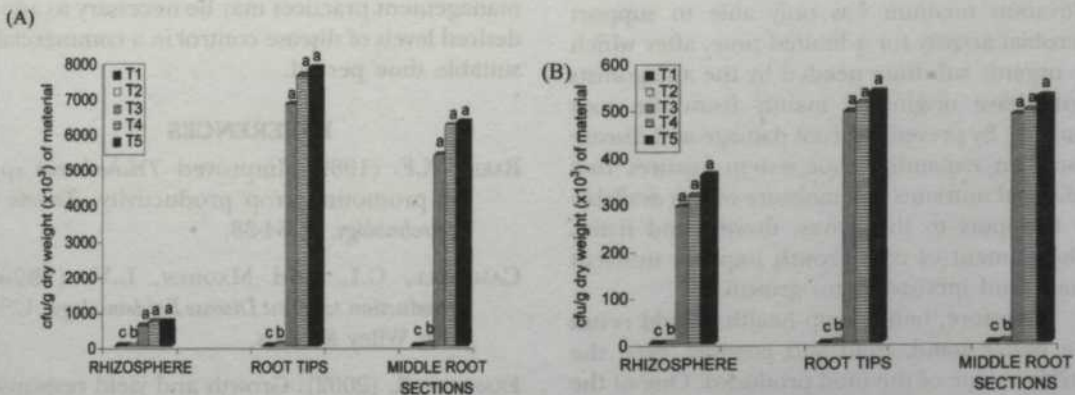


Fig. 3: Frequency of isolation of *Trichoderma* from the rhizosphere, root tips and middle root sections of tomato grown in Fol non-infested (A) and infested soil (B) at 12 weeks after inoculation. Means with different letters within the same tested zone are significantly different using HSD_{0.05}. T1: Control (Sterilised distilled H₂O), T2: Compost alone, T3: UPM 23, T4: UPM 40, T5: UPM 2340

DISCUSSION

This study showed that there was a positive relationship on the effects of *Trichoderma* (UPM 40 and UPM 23) on plant vigor and induced disease suppressiveness. Although there was a reduction in root mass in the presence of the pathogen, *Trichoderma*-induced suppressive soil maintain plant vigor and increased its tolerance to Fusarium wilt.

In recent studies, substrate amendment with *T. harzianum* resulted in enhanced plant growth throughout the growing season (Baker, 1989; Harman and Bjorkman, 1998; Ismail, 2001; Franklin, 2002; Ibrahim, 2005). It also protects the root from certain physical stresses thus allowing the roots to grow faster. Successful colonization of the tomato roots by *Trichoderma* involved the utilization of energy and nitrogen sources present in the root exudates. Movement of *Trichoderma* populations from substrates towards young germinated roots was due to root exudates, which serves as nutrient to *Trichoderma*. Studies by Sariah and Cheng (1999) showed that populations of UPM 23 incorporated into a mixture of coconut dust and peat (1:1 v/v) increased for the first four weeks in the growing medium, then decreased marginally until week 12 because of increased populations of *Trichoderma* detected on roots. Franklin (2002) also observed that *Trichoderma* spp. survived and proliferated in the growing medium consisting mixture of coconut dust and peat and colonized roots of tomato plants as the source of organic matter in the medium was depleted. In addition, Jensen (1997) reported that organic materials in cultivation medium was only able to support microbial activity for a limited time, after which the organic substrate needed by the antagonists would have originated mainly from the root exudates. By preventing root damage and disease attack, an expanded root system ensures that additional nutrients and moisture will be available for transport to the leaves, flowers and fruits. Enhancement of root growth improve nutrient uptake and increase plant growth.

Therefore, better crop health should result in a better stand, yield and possibly, even the nutritive value of the food produced. One of the mechanisms involved in beneficial effect of antagonistic microorganisms was increased uptake of nutrients (N, P and K), which have an important role on plant growth and subsequent

yield. Mao *et al.* (1998) reported that tomato seeds treated with *Burkholderia cepacia* and *Gliocladium virens* individually and in combination significantly increased fruit yield of tomato and pepper in the field compared to the non-treated plants. Maman (2004) also reported that a mixture of *B. cepacia*, *Pseudomonas aeruginosa* 1 and *P. aeruginosa* 2 increased marketable fruit yield of tomato. In this study, application of mixture inoculants (UPM 2340) resulted in a greater yield increase, suggesting that the use of combinations or multiple antagonists may enhance yield and improve disease control over the use of single microorganisms. Such combinations may overcome inconsistencies in the performance of individual isolates. However, there was no significant difference between treatments of UPM 40 and the mixture of UPM 23 and UPM 40 (UPM 2340), suggesting that UPM 40 might have contributed significantly to the biological activity in the mixture.

The phenomena of disease suppressive soils have been documented for numerous plant-pathogen systems. Harnessing the potential of these soils as a practical means to manage diseases in conventional and organic growing conditions has long been a goal of plant pathologists. The findings reported here, demonstrate that the manipulation of microbial communities to induce a disease suppressive soil environment does possess potential as a tool in the management of soilborne plant diseases. The use of specific effective antagonists such as *Trichoderma* spp. can elicit the desired shifts in microbial community structure, but integration with additional management practices may be necessary to attain desired levels of disease control in a commercially suitable time period.

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Flexural Properties of Laminated Veneer Lumber Manufactured from Oil Palm Veneers

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Keywords: Laminated veneer lumber, flexural properties, veneer lay-up pattern, glue-spread rate

ABSTRACT

The uniform properties and higher strength of laminated veneer lumber (LVL) makes it a superior structural material than other solid timber or glue-laminated timber. It requires round logs to process into veneer and laminated parallel to each other to form LVL. However, due to shortage of quality timber (round log), efforts have been made to use alternative materials such as oil palm trunk to produce LVL. This study was undertaken to determine the flexural properties of oil palm LVL of different veneer lay-up patterns and glue-spread rates. In this study, nine types of LVL were produced using three different veneer lay-up patterns and three glue spread rates i.e., 250, 300 and 350 g m² for double glueline. The results revealed that LVL with optimum flexural properties (bending properties and shear strength) was produced using outer-layer veneers bonded by phenol formaldehyde at a glue spread rate of 350 g m². Higher glue spread rate and veneers of the outer-layer of oil palm trunk can produce LVL of better flexural properties.

INTRODUCTION

Laminated Veneer Lumber (LVL) is a structural composite lumber which is produced by many companies around the world. The production technology of LVL has been widely established.

The uniform properties and higher strength of LVL makes it a superior structural material than other solid timber or glue-laminated timber. It requires round logs to process into veneer and laminate parallel to each other to form LVL. However, due to shortage of timber (round log), good quality and larger timber is difficult to find in natural forests of Malaysia. Therefore, efforts have been made to use another alternative material to produce LVL. Many researchers had found that the oil palm trunk can be converted into veneers for producing plywood and converted into sawn timber, particle and fiber for particleboard and medium density fiberboard, respectively (Khozirah *et al.*, 1991; Paridah and Zaidon, 2000). Thus, this provides a potential alternative veneer material for LVL to supplement the depleting supply of timber.

There are extensive oil palm plantations in Malaysia covering a total of 4.2 millions hectares (Bakar *et al.*, 2007). The annual availability of oil palm trunk is estimated to be around 13.6 million

logs based on the 100,000 hectares of replanting reach year (Paridah *et al.*, 2007).

However, oil palm which is a monocotyledonous species does not possess any vascular cambium, so it does not increase in diameter with age. All procambial cells in monocotyledons typically differentiate into primary xylem or phloem, leaving no vascular cambium. These plants, therefore, do not produce secondary xylem and phloem that serves to increase stem diameter throughout the life of the tree. The typical feature is the distinct occurrence of the primary vascular bundles that are randomly embedded in parenchyma ground tissues (Tomlinson, 1961). These vascular bundles are concentrated at the outer portion and reducing towards the inner portion of the trunk. This uneven distribution of vascular bundles along the radial direction of trunk corresponds to a great variation of density values at different parts of the oil palm trunk. The veneer ribbon density distribution along the trunk was found to decrease from outer layers towards inner layers and from bottom part towards the top part of the oil palm trunk. The density values of the oil palm trunk ranged from 200 to 600 kg m⁻³ with an average of 370 kg m⁻³ (Lim and Khoo, 1986).

The veneers peeled from oil palm trunk can be segregated into four density groups i.e., high density (600 kg m^{-3}), medium density ($400\text{-}600 \text{ kg m}^{-3}$), low-medium density ($<400\text{-}550 \text{ kg m}^{-3}$) and low density ($< 400 \text{ kg m}^{-3}$) based on the section of the oil palm trunk (Lim and Khoo, 1986). The high density gradients which exist along the radial, as well as, the longitudinal directions of the trunk indicate that the oil palm trunk is unable to produce consistent quality veneer like other forest timber. Therefore, products from oil palm veneers may lack quality in terms of the flexural strength which is important for structural application.

The objective of this study was to determine the flexural properties i.e., bending properties and gluebond shear strength of LVL produced from oil palm veneer by using different veneer lay-up patterns and glue-spread rates.

METHODOLOGY

The veneers used in the production process were rotary peeled from oil palm trunk in a commercial plywood factory. There were two peeling stages. The first peeling stage peeled to about 25 cm (10 in) in diameter after debarking. Veneer peeled from the first peeling stage was identified as outer-layer veneers having an average thickness of 3.5 mm. For the second peeling stage, the billet was transferred onto a small lathe machine where the trunks were peeled until a final diameter of 12 cm was achieved. These veneers were about 4.1 mm thick and were identified as inner-layer veneers. Each veneer density was determined and recorded. All the veneers were dried to 7% moisture content.

The glue used to laminate the veneer was phenol-formaldehyde adhesive, which gives a bond of exterior grade. The LVL was manufactured with dimensions of 45 mm x 45 mm and a final thickness of 12 mm using 5 plies of veneer at the Faculty of Forestry, Universiti Putra Malaysia.

Nine types of LVL with five replicates each were produced for this study using three different veneer lay-up patterns; a) homogenous pattern of outer-layer veneers, b) homogenous of inner-layer veneers and c) mixture of outer-layer and inner-layer veneers (surfaces using outer-layer and core using inner-layer veneers). Three glue spread rates, 250, 300 and 350 g m^{-2} were used in the manufacture of the LVL.

The assembled veneers were then cold pressed for 5 minutes and hot pressed at 130°C for 15 minutes. The LVLs were conditioned in a conditioning room maintained at a relative humidity of $65 \pm 5\%$ and $20 \pm 20^\circ\text{C}$ for 7 to 10 days prior to evaluating the properties.

A total of 180 bending and gluebond shear samples were cut from the LVL boards with 10 replicates for each type of LVL. The bending properties of the LVL specimens were tested according to BS/EN 310: 1993 (BSI, 1993a). The glue bond shear test was determined according to BS/EN 314-1: 1993 (BSI, 1993b). In addition, dimensional stability tests were also carried out by soaking the test samples in cold water for 24 hours.

All the flexural data collected were analysed using analysis of co-variance (ANOCOV) to determine the interaction between the variables and effect of the variables used in this study on the bending properties and gluebond shear strength of oil palm LVL at 12% moisture content. The means of the bending strength, stiffness and gluebond shear strength were further analysed by the Least Square Means method to determine the oil palm LVL with optimum bending and gluebond shear performances.

RESULTS AND DISCUSSION

Density of Oil Palm Veneers

The total length of the veneer ribbon that can be peeled from the oil palm trunk was about 15 meter. The length of veneer ribbon which can differentiate the outer- and inner-layer is approximately 4 meter. The density of the outer-layer veneers ranged from 358 to 442 kg m^{-3} whereas density of inner-layer veneers ranged from 272-446 kg m^{-3} .

Physical Properties

The average moisture content of the veneers and densities of the LVL boards are shown in Table 1. LVL produced using homogenous outer veneers had the highest density whereas boards produced using inner veneers had the lowest density.

As shown in Table 1, the average thickness swelling of the oil palm trunk ranged from 23.4 to 30.1% while the water absorption ranged from 49.7 to 62.5%. The highest thickness swelling and water absorption percentage was found in LVL manufactured from inner layer

TABLE 1
Moisture content and density of oil palm LVL

Gluespread rate g m ⁻²	Layup Pattern	Moisture content %	Board Density kg m ⁻³	Thickness swelling %	Water Absorption %
250	Outer	11.16	529	24.4	54.3
250	Inner	10.84	520	28.5	62.5
250	Mix	10.86	528	27.1	58.9
300	Outer	11.41	555	23.4	53.1
300	Inner	11.17	505	30.1	57.0
300	Mix	11.16	539	27.3	56.5
350	Outer	10.13	573	28.3	49.7
350	Inner	11.45	511	30.1	54.6
350	Mix	11.75	549	30.0	53.7

veneers using glue spread rate of 350 and 200 g m⁻² respectively. The low density of the inner layer veneers may be attributed to the poor performance of the dimensional stability. Vick (1999) stated that high density timbers normally had thicker cell wall and smaller lumen which associate with smaller volume for water absorption compared to lighter density timbers.

Bending Properties

Bending stiffness of the LVL produced from oil palm was lower than for LVLs produced from tropical hardwood species as reported by H'ng *et al.* (2002). He reported that LVL produced from *kedondong*, *bintangor* and white *meranti* respectively had Modulus of Elasticity (MOE) values ranging from 12,000 to 18,000 N mm⁻² and Modulus of Rupture (MOR) values ranging from 34 to 40 N mm⁻². The maximum MOE values found in oil palm LVL manufactured from outer veneer layer with a glue spread rate of 350 g mm⁻² is far below the minimum requirement for that used in structural applications. According the PRL 501: Performance standard for laminated veneer lumber (APA, 2000), the minimum MOE for

LVL to be used in structural applications is 10392 N mm⁻².

Assessment of the failure pattern of the static bending specimens revealed that almost 80% of the samples failed the compression area as shown in *Fig. 1*. Curry and Fewell (1977) stated that material which failed at the compression area during static bending always associates with lower MOR and MOE because compression failure is an early failure that is found in the bending test before the material can sustain the maximum breaking force.

Analysis of co-variance (ANOCOV) revealed highly significant interactions of glue spread rate and lay-up pattern on the MOR and MOE of oil palm LVL as shown in Table 2.

As shown in Table 2, the highest MOR and MOE were achieved for the LVL produced using outer-layer veneers with glue spread rate of 350 g/m². This may be due to the higher density of LVL produced from the outer-layer. Density is an indicator and predictor for bending strength, stiffness, and various mechanical properties (Panshin and de Zeeuw, 1980). As the board's density increased, the strength properties of the

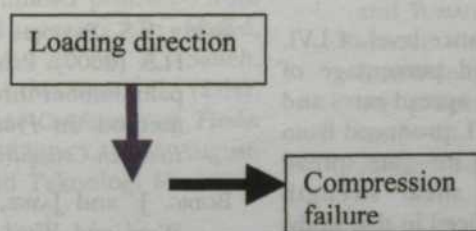


Fig. 1: Compression failure of the oil palm LVL

TABLE 2
Influence of Glue spread rate and lay-up pattern on MOR and MOE

Glue spread rate	Lay-up pattern	MOR N mm ⁻²	MOE N mm ⁻²
250	Outer	30.96 ^{bc} (7.7)	3972 ^c (1028)
250	Inner	25.00 ^{de} (2.4)	2758 ^d (214)
250	Mix	30.39 ^c (3.4)	3762 ^c (688)
300	Outer	34.01 ^b (3.3)	5012 ^{ab} (277)
300	Inner	18.60 ^e (2.5)	1907 ^e (256)
300	Mix	25.96 ^d (2.2)	3723 ^{cd} (484)
350	Outer	39.85 ^a (5.2)	5399 ^a (881)
350	Inner	21.89 ^e (2.9)	2469 ^d (405)
350	Mix	30.64 ^{bc} (3.7)	4815 ^b (479)

Note: Means with the same letter in the same column are not significantly different. ($P < 0.05$)
The values in brackets are standard deviations

board also increased (Bodig and Jayne, 1982). All the LVL manufactured with glue spread rate of 350 g m⁻² was higher in MOR and MOE than the LVL using the glue spread rate of 250 g/m² and 250g/m². Youngquist (1999) stated that adhesive played an important role on the bending strength of LVL. By having an optimum adhesive on laminating the veneers, maximum strength can be obtained from the LVL.

Gluebond Shear Strength

ANOCOV results revealed that the effect of glue spread rate and lay-up pattern on the glue bond shear strength was highly significant. Interaction between the glue spread rate and lay-up pattern was also observed, which means that the shear strength was affected by both glue spread rate and lay-up pattern together.

Table 3 shows the significance level of LVL glue bond shear strength and percentage of wood failure for different glue spread rates and lay-up pattern in this study. LVL produced from outer-layer veneer with 350 g m⁻² glue spread rate had the highest LVL shear strength compared to other LVLs produced in this study. The gluebond shear strength result is consistent

with the findings for the bending properties. By improving the density of the oil palm's LVL using outer-layer veneer and higher glue spread, the glue bond shear strength will increased.

CONCLUSION

LVL with optimum flexural properties (bending properties and shear strength) can be produced using outer-layer veneers which were obtained by peeling the trunk to about 25 cm (10 in) in diameter and bonded by using phenol formaldehyde at glue spread rate of 350 g m⁻².

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TABLE 3

Influence of the glue spread rate and lay-up pattern on glue bond strength and wood failure

Glue spread rate	Lay-up pattern	Glue bond strength N mm ⁻²	Wood Failure %
250	Outer	2.05 ^b (0.51)	80
250	Inner	1.64 ^{bc} (0.13)	100
250	Mix	1.54 ^c (0.26)	100
300	Outer	2.19 ^a (0.15)	90
300	Inner	1.41 ^c (0.11)	80
300	Mix	1.61 ^{bc} (0.21)	80
350	Outer	2.56 ^a (0.39)	90
350	Inner	1.68 ^{bc} (0.25)	80
350	Mix	1.83 ^{bc} (0.31)	80

Note: Means with the same letter in the same column are not significantly different at ($P < 0.05$)
The values in brackets are standard deviations

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A Population Genetics Study on the Malaysian Wild Stocks, *M. rosenbergii* Using Cross Amplified Microsatellite Primers

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ABSTRACT

Distantly related species often develop mutations in priming sites of fast evolving gene sequences (e.g. microsatellite loci) over time. Orthologous loci from green-lipped mussel were found to cross amplify the Malaysian giant freshwater prawn, *Macrobrachium rosenbergii* microsatellites, which indicates a high conservation of flanking sequences. This was confirmed by direct sequencing of target loci. Four microsatellite markers were successfully cross-amplified in the 'western' form of *M. rosenbergii* with two containing minor interruptions in their sequences. Number of alleles per locus ranged from 5 to 8 with allele frequencies ranging from 0.0048 to 0.2656. Observed heterozygosities ranged from 0.4369 to 0.6848, but in general, these estimates were lower than expected under Hardy-Weinberg equilibrium. Genetic distance values ranged between 0.0059 and 0.1641 with populations in the UPGMA dendrogram not clustering according to their geographical localities.

INTRODUCTION

Prawns of the genus *Macrobrachium* Bate, 1868 (Crustacea: Palaemonidae) are a highly diverse, abundant and widespread group of decapod crustaceans found in circumtropical marine-, estuarine- and freshwaters (de Bruyn *et al.*, 2004). The giant freshwater prawn, *Macrobrachium rosenbergii*, is the largest species in the genus *Macrobrachium* and the most important culture species (Mather and de Bruyn, 2003) It is found in coastal river systems from Pakistan in the west to Vietnam in the east, throughout Southeast Asia and south to northern Australia and Papua New Guinea (de Bruyn *et al.*, 2004).

M. rosenbergii as it is currently named taxonomically may be polytypic both regionally and within biogeographical regions (de Bruyn *et al.*, 2004). Two distinct forms of *M. rosenbergii*, an 'eastern' and a 'western' form have been described independently, although the species is currently considered to be monophyletic (Mather

and de Bruyn, 2003; de Bruyn *et al.*, 2004; Chand *et al.*, 2005).

Microsatellites are a popular marker of choice in many population studies and have been applied in gene mapping, forensics and behavioural ecological studies (Goldstein and Schlotterer, 1999). However, single locus microsatellite marker development can be tedious, laborious, time consuming, costly and requires specialised facilities and equipments. Conservation of flanking regions surrounding microsatellites had been reported across taxa in many animal species (Schlotterer *et al.*, 1991), which facilitates cross-species amplification in a new target species. Recently, six specific microsatellite primer sets were developed for the 'eastern' form of giant freshwater prawn, *M. rosenbergii*, and the variation characterized (Chand *et al.*, 2005). However, cross amplification of these loci in the related 'western' form of the species was unsuccessful although this form is by

far the most important with respect to worldwide wild fisheries and culture fisheries.

The homologous sequences that existed between two families, *Palaemonidae* and *Mytiloidea*, was very interesting when BLAST analysis performed on RAMS clones of *M. rosenbergii* showed that a number of the sequences were homologous to *Perna viridis* (Bhassu *et al.*, 2005). Thus, microsatellite primers developed from a mollusc, the green-lipped mussel (*Perna viridis*) were evaluated for their potential to cross amplify in Malaysian populations of the 'western' form of giant freshwater prawn. In the gene bank, there are more than 100 microsatellites sequences of *Perna viridis* that can be further tested for its potential for cross amplification in *M. rosenbergii*. Similar studies on marine turtles indicated conservation of flanking sequences spanning approximately 300 million years of divergent evolution (FitzSimmons *et al.*, 1995). Ohno (1970) proposed that without duplicated genes, the creation of metazoans, vertebrates and mammals from unicellular organisms would have been impossible.

It proves that microsatellite loci have great potential for broader applications such as

comparative gene mapping and assessing genetic population structures within species. The use of microsatellite loci across species depends on the conservation of priming sites within flanking sequences, which enables amplification and maintenance of repeat arrays long enough to promote polymorphism (Weber, 1990).

The objective of this study was to identify potential microsatellites to be used for cross amplification in *M. rosenbergii* and to aid in finding potential polymorphic microsatellites for use in marker assisted selection programs.

MATERIALS AND METHODS

Samples and DNA Extraction

Macrobrachium rosenbergii were sampled from a total of 11 rivers representing a diverse geographical distribution consisting of 8 populations from east and west Peninsular Malaysia and two populations from the island of Borneo in Sarawak and Sabah (Fig. 1). The sample sizes are shown in Table 1. DNA was extracted from 25 mg of tissue following the recommended protocol in QIAamp DNA Mini Kit (Qiagen, Germany).

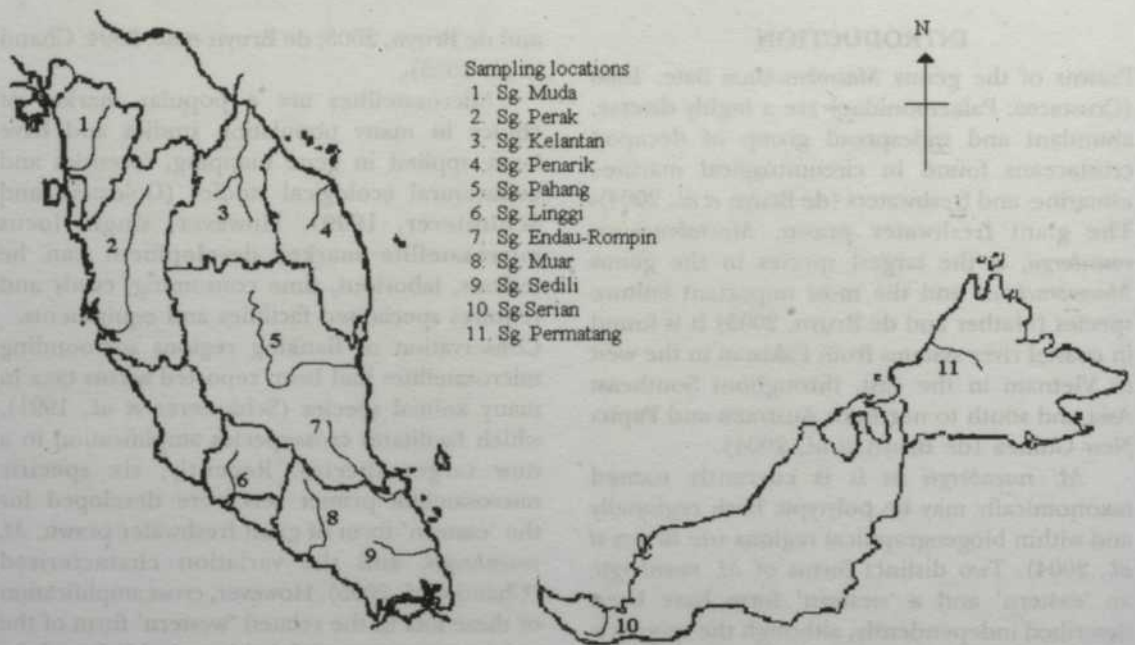


Fig. 1: Location of sampling sites

TABLE 1
 Sampling sites and sample sizes for 11 populations

No.	Location	Sample size
1	Sungai Perak, Perak	20
2	Sungai Muda, Kedah	20
3	Sungai Muar, Johor	20
	Sungai Sedili, Johor	20
4	Sungai Endau, Johor	20
5	Sungai Linggi, N.Sembilan	30
6	Sungai Pahang, Pahang	30
7	Sungai Kelantan, Kelantan	30
8	Sungai Penarik, Terengganu	30
9	Sungai Serian, Sarawak	20
10	Sungai Permatang, Sabah	20

Microsatellite Screening and Amplification

Twelve *Perna viridis* microsatellite primers were screened on individuals of *M. rosenbergii* by polymerase chain reaction (PCR). PCR products were electrophoresed and visualized under ultraviolet light. Successfully amplified bands within the expected size range were excised from the gel. Gel excision and purification were performed using the Perfectprep/EGel Cleanup Kit (Eppendorf, Germany).

Excised bands were sequenced in ABI PRISM 377 DNA sequencer using BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, USA) to determine the amplification of microsatellite repeats in the sequence. This process resulted in four primer sets that amplified apparent microsatellites containing fragments in giant freshwater prawn samples.

Population Characterization

Based on the screening procedure, four *Perna viridis* microsatellite primer sets that amplified fragments in giant freshwater prawn, OCC26, OCC28, OCC34 and OCC43, were tested in individuals sampled across a large geographical range in Malaysia. The study was carried out by PCR amplification using a Biometra T3 Thermal Cycler. Thermal cycle amplification was performed in 10 μ L total reaction volume, which contained 1x PCR buffer (Promega, USA), 2.5 mM to 3.75 mM of MgCl₂ (Promega, USA), 0.2 unit of *Taq* polymerase (Promega, USA), 0.25 mM of each dNTPs (Promega, USA), 50 ρ mole of primer pair, deionised water and approximately 20 ng of template genomic DNA.

The general PCR profile consisted of 40 cycles of 94 °C denaturation for 30 s, 30 s at an annealing temperature specific for each primer pair (as shown in Table 2) and 72 °C of elongation for 40 s. The PCR profile was initiated with a 3 min incubation at 95 °C before the cycle began and upon the completion of the cycles, a 5 min incubation at 72 °C was performed. PCR products were electrophoresed on 6% non-denaturing polyacrylamide gels. Sizes of alleles were determined according to M13 sequence ladder.

Data Analyses

The number of alleles per locus, number of effective alleles per locus, and observed and expected alleles per locus were calculated. Conformity to Hardy Weinberg equilibrium was tested using the Markov Chain Method (dememorization: 1000, batches: 500 and iterations per batches: 1000). All these calculations were performed using the CENEPOP computer package (Raymond and Rousset, 1995). The independent t-test comparison was used to check the differentiation of observed heterozygosity among populations (Archie, 1985).

The GENEPOP software package was used to calculate F-statistics (F_{IS} , F_{ST}) test of genetic disequilibrium for each pair of loci, and genetic differentiation between populations (dememorization: 100, batches: 100 and iteration per batches: 1000). Due to the small number of loci used, the software package did not allow for significant tests for overall F_{ST} . The locus-wise F_{IS} for each population was also calculated to detect effects of inbreeding and Wahlund effects.

Genetic distance between populations was based on the Cavalli-Sforza and Edwards (1967) chord distance. The unweighted pair group method with arithmetic averaging (UPGMA) tree including boot-strap values was constructed as dendrograms with the SAHN (sequential, agglomerative, hierarchical and nested clustering) program from NTSYS-pc software version 1.60 (Numerical Taxonomy and Multivariate Analysis System) (Rohlf, 1993). Mantel's test was performed to evaluate

correlation between genetic distances and geographical distances (Manly, 1993 using TFGA version 1.3 (Miller, 1997)

RESULTS AND DISCUSSION

Two of the *Perna viridis* microsatellite primer pairs that amplified in *M. rosenbergii* resulted in products, which contained uninterrupted microsatellite sequences, VJ1-1-1 and BP2-49-2 while 2 loci contained minor interruptions of the sequences, VJ1-9-1 and VJ1-11-2. The

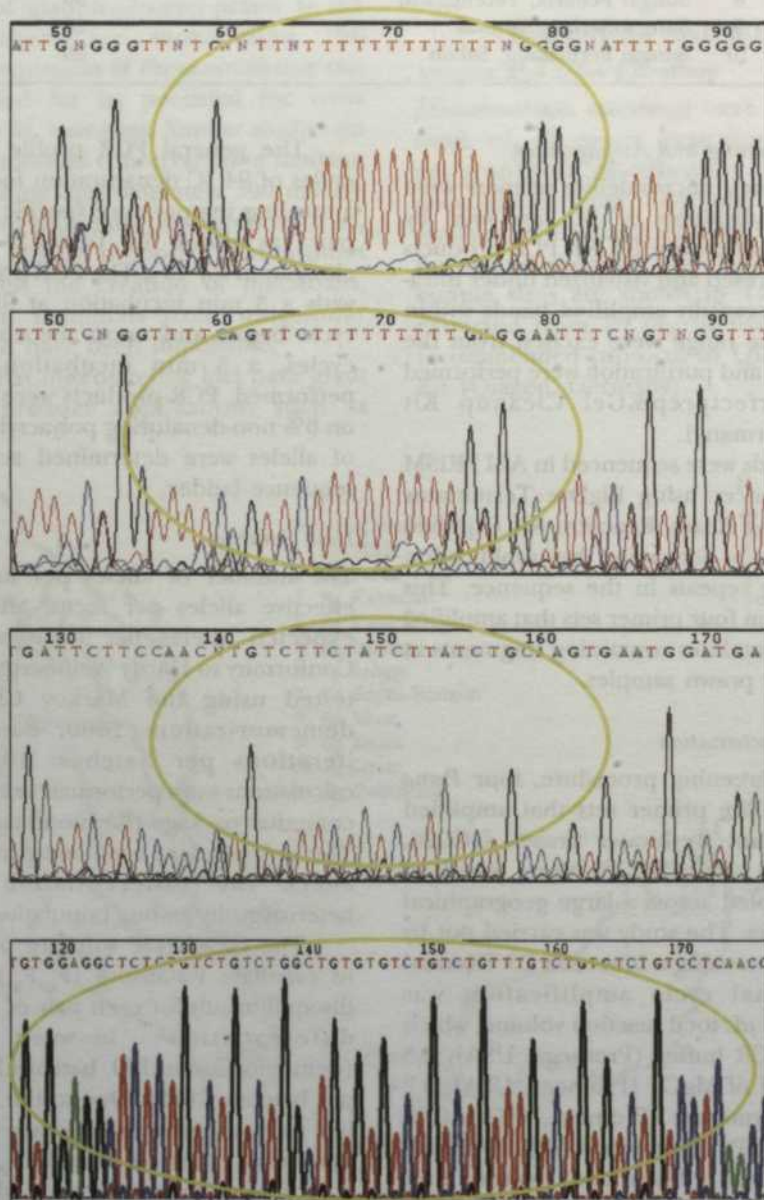


Fig. 2. Microsatellite regions amplified in *M. rosenbergii*. (a) VJ1-11-2; (b) BP2-49-2; (c) VJ1-1-1; (d) VJ1-9-1

amplified flanking sequences are shown in Fig. 2. These results suggest cross-amplified microsatellite markers in *M. rosenbergii*.

Microsatellite typing of wild 'western' *M. rosenbergii* populations sampled from Malaysian rivers indicated that all the four loci were polymorphic. Characterization of the four polymorphic loci and a comparison of the repeat sequences between the two species are summarized in Table 2. The number of alleles per locus ranged from 5 to 8 with the overall allele frequency ranging from 0.0042 to 0.2549 (Fig. 3). VJ1-1-1 was found to have the highest percentage of effective number of alleles (90.85%), followed by BP2-49-2 (83.45%), VJ1-9-1 (82.62%), VJ1-11-2 (80.69%).

Polymorphisms among the 11 populations was highest for VJ1-11-2, followed by VJ1-1-1, BP2-49-2 and VJ1-9-1. The expected size of the alleles are shown in Table 2. Null alleles were present in few individuals of the Kelantan populations, which could be due to possible mutations at the flanking region of the loci. Studies conducted by Bhassu *et al.*, (2007) indicated that allele at 475 bp of Random Amplified Microsatellite Marker (RAMs) serves as a diagnostic marker in identifying Sg Kelantan samples from the other populations. This could indicate the presence of cryptic species within *Macrobrachium sp.* However, morphology and mt-DNA analysis should be carried out to reconfirm the postulation.

The observed heterozygosity ranged from 0.4421 to 0.7851 (Table 3), which was generally lower than the expected heterozygosity. Such results could be explained by several hypotheses, including methodology bias, null alleles (Jarne and Lagode, 1996) and 'founder effect' during the introduction (Mei *et al.*, 2003).

The F_{IS} values for each population across all loci (Table 3) indicated heterozygote deficiency ($P < 0.05$) for all. A comparison of the observed and expected variation indicated that all the loci deviated from Hardy-Weinberg expectations ($P < 0.05$), which might have resulted from the small sample sizes (20 - 30 individuals) analysed per population or the presence of null alleles and high number of alleles. However, three populations, namely Muar, Sedili and Pahang were found not to have deviated from HW equilibrium at locus VJ1-1-1 marker while Kedah did not deviate from HW equilibrium at locus VJ1-11-2. There was no significant linkage

disequilibrium between each pair of loci within each of the eleven populations. ($P < 0.05$). An independent t-test (Archie, 1985) showed that neither observed nor expected heterozygosity was significantly different between populations.

F_{ST} was 0.062 and significantly different from 0 (Table 4), thus clearly showing that populations of *M. rosenbergii* are divided into subpopulations. The small magnitude of differentiation may be the result of small sample size constraints and large number of alleles, thus increasing the sample size creates high statistical error.

Genetic distance (Cavalli-Sforza and Edwards (1967)) based on the four loci revealed average values between 0.0059 and 0.1641 (Table 5). The dendrogram (Fig. 4) showed that the majority of the populations did not cluster according to their geographical location, which may be caused by natural selection and mutation in order to adapt to a new environment. Mantel's test showed no significant correlation between genetic and geographical distances ($r = 0.1669$; $Z = 1600409.6635$; upper tail $P = 0.2360$; lower tail $P = 0.8452$)

CONCLUSIONS

This approach compared to conventional methods is indeed cost effective as it reduces the time taken to isolate microsatellite markers. It utilizes *Perna viridis* microsatellite primers on *M. rosenbergii* stocks for genetic diversity, mutational mechanism, phylogenetic and other studies. Thus more cross-amplified microsatellite primers should be trailed as this study unexpectedly yielded positive results. The dendrogram generated suggested that the clustering of the populations were not exactly in accordance with their geographical locations, which might be due to natural selection in order to adapt to new environments and their breeding systems. The low genetic variation in the eleven populations may have been due to the small sample size and low number of loci used. Therefore there is need to increase the number of loci and sample size. The potential of *Perna viridis* primers will be studied further using large sample populations and at least 20 loci. Distinct genetic differentiation suggested that these populations should be managed separately. Moreover, genetically differentiated populations may show variation of traits important for aquaculture (Kumagai *et al.*, 2004). Therefore economically

TABLE 2

Characteristics of *Perna viridis* microsatellites in *M. rosenbergii*, including GeneBank Accession number (*Perna viridis*), primer sequence, repeat sequence, annealing temperature (T_A), expected size (bp) (*Perna viridis*) and size range (bp) (*M. rosenbergii*)

Note: N – Uninterrupted repeat
n – Interrupted repeat

Primer	GeneBank Accession No. (<i>Perna viridis</i>)	Primer Sequence (5' – 3')	Repeat Sequence (<i>Perna viridis</i>)	Repeat Sequence (<i>M. rosenbergii</i>)	T_A (°C)	Expected size (bp) (<i>Perna viridis</i>)	Size range (bp) (<i>M. rosenbergii</i>)
VJ1-1-1	DQ010069	F: CAC CTA GTT CAG GGT CTC TC R: AGC TCT CAT CCA TTC ACT TG	(TC) _N	(TCT) _n	44	174	143-276
VJ1-9-1	DQ010072	F: TGC GTG TGG AGG CTC TCT R: TCA CCT CTT GGT TGA GGA CA	(CT) _N	(TCT) _N	40	205	151-257
VJ1-11-2	AY850125	F: ACT CGA TCT CTG TGT TGT TA R: TAG TTT CAG GTT CAC TAT GG	(TG) _N	interrupted (G) _N (T) _N	44	234	144-220
BP2-49-2	AY850129	F: GTT AAA CAA CCA ACC AAC G R: GTC TTT TTG TCA TTG CAC AC	(TG) _n	(T) _n	44	215	122-290

A POPULATION GENETICS STUDY ON THE MALAYSIAN WILD STOCKS

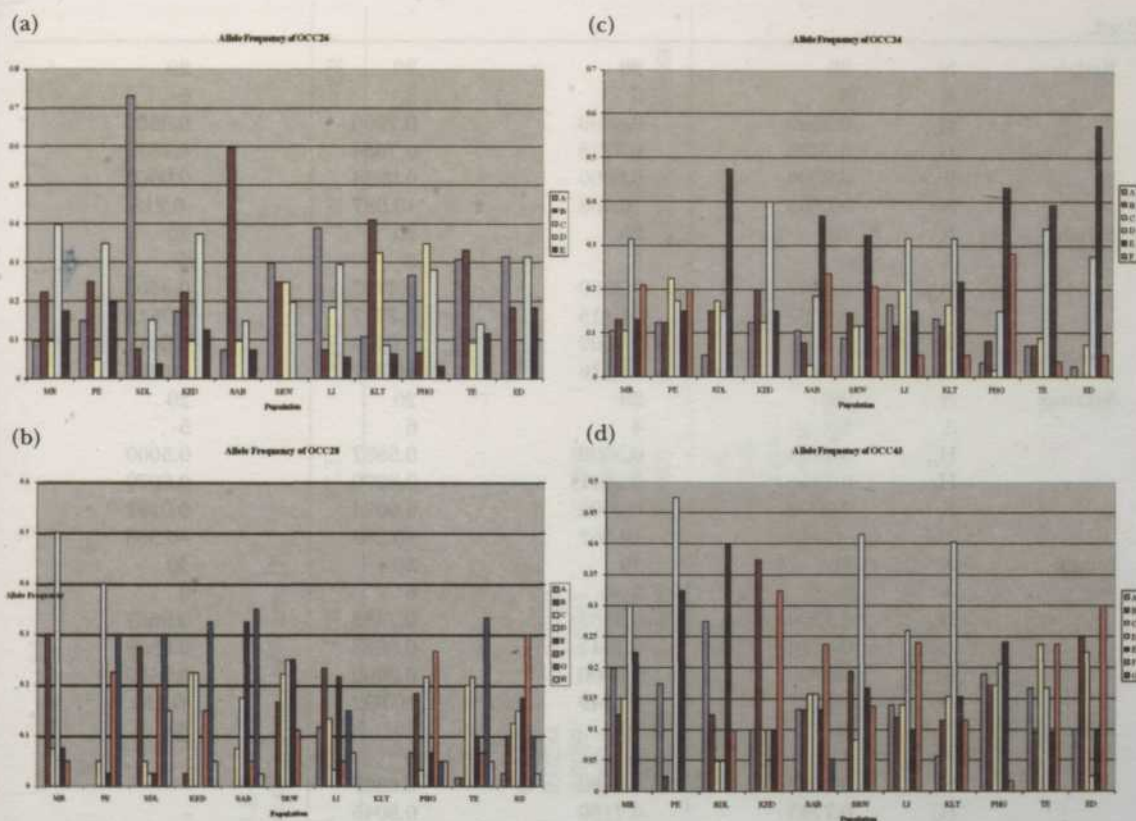


Fig. 3: Allele frequencies within the 11 populations. (a) VJ1-1-1; (b) VJ1-9-1; (c) VJ1-11-2; (d) BP2-4912

TABLE 3
Statistical results of *Perna viridis* microsatellite in *M. rosenbergii* populations

Locus		VJ1-1-1	VJ1-9-1	VJ1-11-2	BP2-49-2
Muar	N	20	20	20	20
	A	5	5	6	5
	H _O	0.7000	0.5500	0.6316	0.7000
	H _E	0.8013	0.7577	0.8208	0.6628
	P	0.0195	0.0000	0.0011	0.7000
	F _{is}	+0.129	+0.279	+0.235	-0.058
Perak	N	20	20	20	20
	A	4	5	6	5
	H _O	0.4000	0.5000	0.6500	0.9500
	H _E	0.6538	0.7692	0.8462	0.7141
	P	0.0002	0.0007	0.0220	0.0001
	F _{is}	+0.394	+0.356	+0.236	-0.342
Sedili	N	20	20	20	20
	A	6	4	5	6
	H _O	0.8500	0.0769	0.3500	0.1500
	H _E	0.7526	0.4523	0.7141	0.7885
	P	0.0566	0.0002	0.0000	0.0000
	F _{is}	-0.133	+0.836	+0.516	+0.814

Table 3 Cont.

Cont.

Kedah	N	20	20	20	20
	A	5	5	5	6
	H _O	0.3500	0.4000	0.7000	0.9500
	H _E	0.7423	0.7718	0.7654	0.7872
	P	0.0000	0.0000	0.0224	0.0002
Sabah	F _{is}	+0.535	+0.488	+0.087	-0.213
	N	20	20	20	20
	A	6	5	6	6
	H _O	0.6842	0.3000	0.4737	0.4500
	H _E	0.8620	0.6115	0.7767	0.7513
Sarawak	P	0.0068	0.0009	0.0009	0.0012
	F _{is}	+0.211	+0.516	+0.397	+0.407
	N	20	20	20	20
	A	5	4	6	5
	H _O	0.4444	0.5000	0.5882	0.5000
Linggi	H _E	0.7556	0.7641	0.8200	0.8079
	P	0.0002	0.0000	0.0094	0.0191
	F _{is}	+0.419	+0.352	+0.289	+0.388
	N	30	30	30	30
	A	6	5	6	8
Kelantan	H _O	0.5200	0.3333	0.7333	0.9667
	H _E	0.8278	0.7317	0.8068	0.8508
	P	0.0000	0.0000	0.0000	0.0409
	F _{is}	+0.377	+0.549	+0.092	-0.139
	N	30	30	30	30
Pahang	A	6	5	6	-
	H _O	0.2692	0.2609	0.6667	-
	H _E	0.7745	0.7150	0.8045	-
	P	0.0000	0.0000	0.0001	-
	F _{is}	+0.657	+0.640	+0.174	-
Terengganu	N	30	30	30	30
	A	6	5	6	8
	H _O	0.5238	0.5238	0.4286	0.9667
	H _E	0.8328	0.7677	0.7240	0.7977
	P	0.0009	0.0000	0.0000	0.0000
Endau Rompin	F _{is}	+0.377	+0.323	+0.413	-0.216
	N	20	20	20	20
	A	6	4	5	8
	H _O	0.9500	0.8947	0.1500	0.9000
	H _E	0.7962	0.7525	0.6000	0.8410
	P	0.0068	0.0000	0.0000	0.0000
	F _{is}	-0.199	-0.195	+0.755	-0.072

Note: N - Number of samples, A - Number of alleles, () - Effective number of alleles, H_O - Observed Heterozygosity, H_E - Expected Heterozygosity, P - Probability value estimates regarding deviation from Hardy-Weinberg equilibrium, F_{st} - Population Differentiation, F_{is} - Inbreeding Coefficient. * Significant Deviation at P=0.05

TABLE 5

Matrix of genetic and geographical distances. Above diagonal is geographical distance (km), below diagonal is genetic distance according to Cavalli-Sforza and Edwards (1967) chord distance

pop ID	MR	PRK	SDL	KDH	SBH	SRW	LNG	KLT	PHG	TRG	ER
MR	0.0000	444	197	593	2389	1989	139	652	281	544	210
PRK	0.0274	0.0000	638	149	2455	2025	345	341	401	453	608
SDL	0.1641	0.1418	0.0000	787	2583	2185	369	660	345	492	92
KDH	0.0764	0.0865	0.1228	0.0000	2604	2204	494	351	550	463	757
SBH	0.0960	0.0821	0.1504	0.0691	0.0000	2000	2290	2674	2383	2655	2553
SRW	0.0369	0.0515	0.1098	0.0758	0.0492	0.0000	1890	2274	1983	2255	2153
LNG	0.0615	0.0803	0.0692	0.0436	0.0847	0.0235	0.0000	564	231	503	321
KLT	0.0285	0.0399	0.1624	0.0704	0.0274	0.0059	0.0353	0.0000	440	168	568
PHG	0.0500	0.0717	0.0756	0.0947	0.0949	0.0217	0.0540	0.0669	0.0000	272	253
TRG	0.0758	0.0655	0.0737	0.0227	0.0165	0.0398	0.0348	0.0212	0.0645	0.0000	401
ER	0.1008	0.1225	0.0758	0.0457	0.0670	0.0571	0.0553	0.1107	0.0502	0.0233	0.0000

MR	- Muar
PRK	- Perak
SDL	- Sedili
KDH	- Kedah
SBH	- Sabah
SRW	- Sarawak
LNG	- Linggi
KLT	- Kelantan
PHG	- Pahang
TRG	- Terengganu
ER	- Endau Rompin

important traits of this population such as growth, disease resistance, and others should be evaluated. Incorporating information on genetic information would enable efficient exploitation of these resources for aquaculture and genetic improvement programs.

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