Dormancy and Cardinal Temperatures during Seed Germination of Five Weedy Rice (*Oryza* spp**.**) Strains

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ABSTRACT

Temperature during seed imbibition has been found to influence germination rate and final percent germination. Seeds of one cultivated variety and five weedy rice strains, collected from different localities in Peninsular Malaysia, were used to determine their degree of dormancy and cardinal temperatures. Meanwhile, standard germination and tetrazolium chloride (TTC) tests were used to evaluate the percentage of seed viability and degree of dormancy. Seed germination test at six different constant temperatures (between 10 and 35°C) was applied to determine the cardinal temperatures estimated by linear regression models, base temperature, T_b, optimum temperature, T_o, and maximum temperature, T_c. The TTC test was found to be a simple and quick test to determine the degree of seed dormancy among different weedy rice strains, when used together with a standard germination test. Germination rate was found to be related to the degree of dormancy but it had no influence on the range of cardinal temperatures. The T_b among the five weedy rice strains was in the range of 2-7.3^{o}C. The T_o varied between 28.1 and 37.5^{o}C, with an average of 32.5^{o}C. This temperature (T_o) was higher than that of the cultivated MR73 variety (24.3^{o}C), whereas the range of T_c was 42.2-43.3^{o}C. The study indicated that the non-dormant cultivated rice seed had lower T_b and T_o values than the dormant seed of weedy rice.

Keywords: Weedy rice, germination, seed dormancy, cardinal temperatures

INTRODUCTION

Weedy rice (*Oryza* spp.) infestation occurs in most major rice-growing areas of the world. The invasive nature of weedy rice has become a major concern, especially in direct seeded rice cultivation. In Malaysia, the presence of weedy rice in a direct-seeded field can reduce yield up to 74% (Bakar *et al.*, 2000). Early shattering has enabled them to escape harvest, while seed dormancy ensures their survival in the soil seed bank. The degree of infestation through soil seed bank germination varies between production years. Nonetheless, the reasons behind these observations are not known. Thus, determining the temperature range at which weedy rice seed germinates will help to predict seedling emergence.

Temperature is an important single factor affecting the capacity for germination by regulating dormancy, and it also critically determines the rate of progress toward completion of germination once a seed is stimulated (Alvarado and Bradford, 2002; Bradford, 2002). The degree of seed dormancy influences the temperature range, at which seed will germinate, with the range increasing as seeds loose dormancy (Benech-Arnold *et al.*, 2000; Vegis, 1964). These critical temperatures,

which are commonly referred to as cardinal temperatures, consist of the base and maximum temperatures, below or above which germination will not occur, while the optimum temperature is where germination is the most rapid (Bradford, 2002). This concept was initially proposed on the whole plant basis, but it is also applicable to seed during germination. Bewley and Black (1994) described cardinal temperatures as the range of temperatures over which seeds of a particular genotype could germinate. In cultivated rice varieties, a high percentage of germination is attained in two days at 27-37°C, while no germination is found to occur at 8°C and 45°C (Yoshida, 1981). However, the cardinal temperatures for seed germination have never been reported in weedy rice strains, which are helpful for predicting the degree of seed dormancy and infestation in the field.

Models which describe seed germination behaviour, in response to a range of temperatures during imbibition, have been proposed and developed (Covell *et al.*, 1986; Ellis and Butcher, 1988; Alvarado and Bradford, 2002; Hardegree, 2006). The model commonly known as the thermal time model has been extensively used and successfully applied to describe germination timing and seedling emergence in crops (Finch-Savage and Phelps, 1993) and weed species (Roman *et al.*, 2000). This particular thermal time model predicts germination rate at sub-optimal temperatures (from the minimum temperature to the optimum temperature) and supra-optimal temperature (i.e. from the optimum temperature to the maximum temperature) in a linear function (Hardegree, 2006). The thermal time model has also been successfully used to predict weeds seedling emergence in the field under temperate growing conditions (Forcella *et al.*, 2000; Vleeshouwers and Kropff, 2000).

Natural selection on germination responses to seasonal environmental cues in some species has been proposed as a significant determinant for the genotype to establish in a given seasonal environment (Donohue, 2005). However, little information is available on the germination responses of weedy rice strains to temperature during imbibition, and there has been no record of its cardinal temperatures to date. Thus, understanding the variation in temperature during seed imbibition may establish germination responses of weedy rice strains seedling emergence in the field. The aims of this study were to (1) determine the cardinal temperatures, base (T_b), optimum (T_o) and maximum (T_c) of the different weedy rice strains, and (2) determine the germination rate within these cardinal temperatures.

MATERIALS AND METHODS

Plant Material

For the purpose of this study, five weedy rice strains and one cultivated variety were used. Seeds of the cultivated variety MR 73 were obtained from the Malaysian Agriculture Research and Development Institute (MARDI). The seeds of five weedy rice strains were randomly collected from several locations in Malaysia, namely Seberang Perak, Kuala Pilah, Besut, Perlis, and Kemubu Agricultural Development Authority in Peninsular Malaysia. Hereafter, these five strains are termed as SP, KP, Besut, Perlis and KADA strains, respectively. The seeds were collected in January and February 2008 and stored at 0°C in double sealed plastic bags for two months before conducting the study. Seed moisture was kept in the range of 9-11% prior to storage and at the start of the experiments.

Seed Viability Tests

For standard germination test, the imbibing seeds were left at ambient temperature in the laboratory at 25±3°C. The seeds were germinated on double layered moistened (±10mL distilled water) filter papers (Whatman, no. 1, in 80 mm diameter by 10 mm deep disposable plastic Petri dishes. The seeds were soaked in 10% Clorox for surface sterilization for 3 min. prior to the testing.

Seedling evaluation on the number of germinated seeds was done daily, starting on day 2 after imbibition for 14 days. Seeds which did not germinate after 14 days were considered as

dead seeds. In contrast, seeds were considered as germinated when the radical emergence was >5 mm. The test was replicated twice with 50 seeds per replication.

For the tetrazolium chloride test, two replicates of 50 seeds per replicate were imbibed in distilled water at room temperature for 24 h. The middle portion of the seeds was pierced with a needle before soaking them in 0.1% of 2, 3, 5-triphenyl tetrazolium chloride (TTC) salt solution. The seeds imbibing in the TTC solution were exposed to 35°C in the oven for 2 h. Viable seed was evaluated based on the topographical staining pattern on the embryo, as described in the ISTA procedure (ISTA, 1993).

Seed Germination Test at Different Temperature

A germination test at different temperatures for all the seeds was conducted in the germination chamber in darkness. Nonetheless, the preliminary works did not show any seed sensitivity to light during germination (Rosli, 2008). Meanwhile, the preparation of seeds and imbibition media are similar to the procedures used for the standard germination test, as described above. The imbibing seeds were exposed to the constant temperatures of 10, 15, 20, 25, 30, or 35°C. This experiment was performed in two replications consisting of 50 seeds per replicate. Seeds were considered as germinated when the radicle was >5 mm. The evaluation was done daily for 20 days, beginning on Day 2 after sowing.

Statistical and Data Analysis

All the collected data were subjected to the analysis of variance using the Statistical Analysis System (SAS) Software, version 8.2. When ANOVA indicated a significant effect, the least significant difference (LSD) was performed to determine significant differences among the means of the treatments.

Meanwhile, the germination rates were calculated as the inverses of times to radicle emergence (Alvarado and Bradford, 2002).

The reciprocals of the time to germination were plotted to estimate the optimum temperature, at which the rate of germination was maximum (T_o). The rates of germination were also subjected to the linear regression analysis to describe cumulative germination response of temperature (SAS Institute, 2005). The cumulative percentage germination (CGP), obtained from the germination tests at different temperatures, were used to calculate the cardinal temperatures. Intersected-line models were used as proposed by Garcia-Huidobro *et al*. (1982). The equation used to describe the rates of germination between base and up to optimum temperatures is as follows:

$$1/t = (T - T_b)/\theta_1 \qquad (1)$$

In order to describe the germination responses above T_o, but below the maximum temperature (T_c), equation (2) was used:

$$1/t = (T_c - T)/\theta_2 \qquad (2)$$

where t is the time taken in days for the CGP to reach a given percentage, T is the temperature, while T_b, T_o and T_c are the base, optimum and maximum temperatures, respectively. These models predict the germination rate for a given seed fraction (sub-optimal and supra-optimal range) in a linear function of temperature. The intercepts of the fitted linear regression lines on the temperature axes were used to estimate T_b and T_o. T_o was calculated as the intercept of sub-optimal and supra-optimal temperature function (Hardegree, 2006).

RESULTS

Viability and Degree of Dormancy

Based on the standard germination test, the initial quality of the five weedy strains was in the range of 19-86% (Table 1). The germination percentage of the cultivated variety (MR73) was found to be the highest (98%). Meanwhile, the lowest germination percentage among all the weedy rice strains was observed in the KADA strain and the highest was in the SP strain, with 19% and 86%, respectively.

The TTC test used was to determine the percentage viability, as well as the degree of dormancy among the weedy strains and for MR 73. The highest percentage of viability was also recorded in MR73 with 99% (Table 1). This suggests that no seed dormancy is present in this particular cultivated variety. Based on the TTC test, a higher percentage of seed viability was observed in all the weedy rice strains, except for the SP strain, as compared to the percentage of viability based on the standard germination test. Based on the TTC test, the viability percentage was found to be 70-83%. Based on this test, the seed of SP strain was not dormant. As for Besut and Perlis strains, the seeds appeared to have a slight dormancy. The data indicate that there is a variation in the degree of seed dormancy among the weedy strains used in this study.

TABLE 1
Percentage viability of the cultivated rice variety and weedy rice strains based on the standard germination and tetrazolium chloride (TTC) tests

Strain/Variety	Cumulative germination (%)	TTC (%)
MR 73[†]	98	99
Seberang Perak	86	83
Kuala Pilah	51	76
KADA	19	74
Besut	65	71
Perlis	74	80
LSD	10	2

[†] cultivated rice variety

TABLE 2
The germination percentage of the cultivated rice variety and weedy rice strains at constant temperatures

Variety/strain	Percentage germination					
	10°C	15°C	20°C	25°C	30°C	35°C
MR 73[†]	58	78	94	98	94	29
Seberang Perak	8	10	54	62	82	54
Kuala Pilah	20	32	50	54	60	34
KADA	0	0	0	8	22	12
Besut	0	0	34	28	48	22
Perlis	6	8	20	24	50	36
LSD	26.2	16.6	17.0	14.1	20.2	19.6

[†] cultivated rice variety

Germination and Germination Rate at Constant Temperatures

The increase in the temperature (i.e. from 10°C to 30°C) during imbibition enhanced the germination percentage of both the cultivated rice variety and weedy strains (Table 2). All the weedy rice strains were found to have low germination percentages ($\leq$82%) as compared to MR73 (>90%) within the range of constant temperatures. Increasing the imbibition temperature to >30°C was found to cause a rapid decline in the germination percentage of both the cultivated variety and weedy strains. Meanwhile, the maximum germination percentage of the cultivated variety was observed at 25°C, whereas this was observed at 30°C for all the weedy rice strains. No germination was observed in KADA weedy strain at temperature 20°C and below, indicating that this strain is highly dormant.
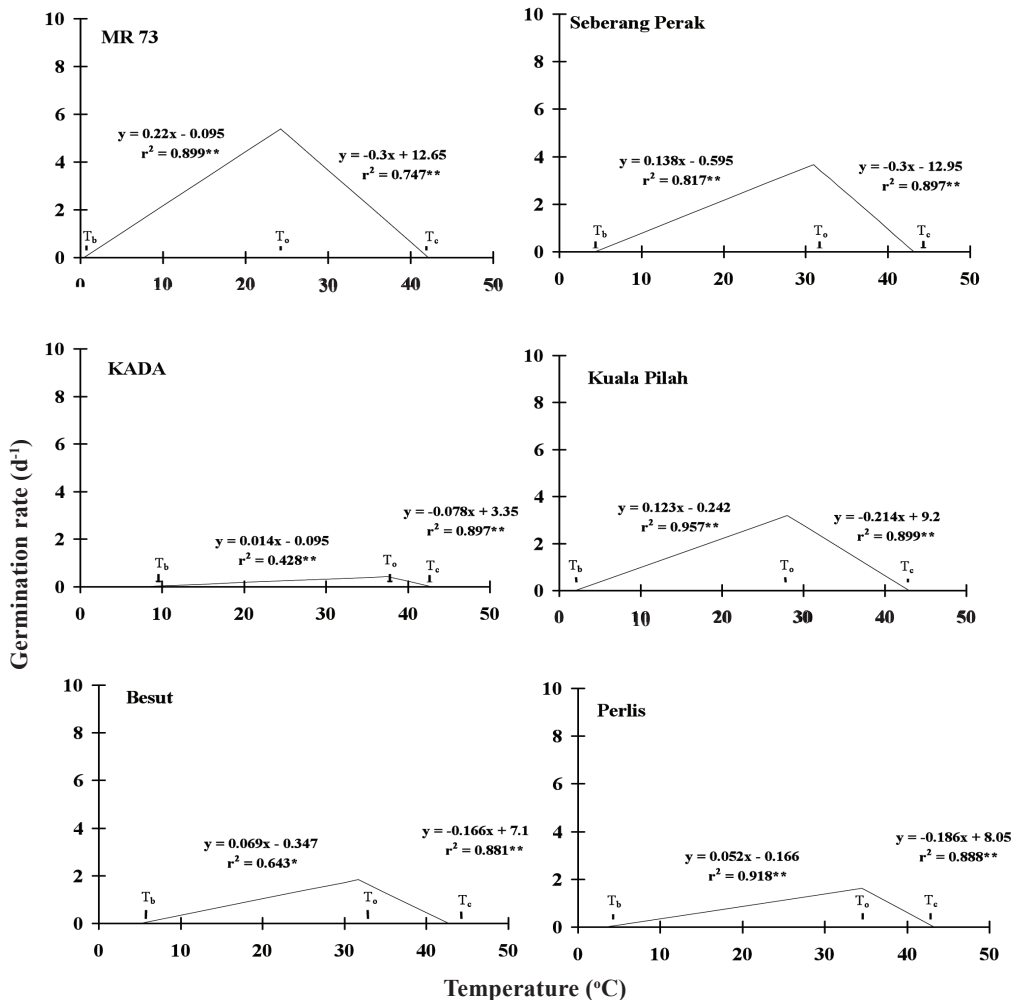

*Fig. 1: Germination rates at the suboptimal and supraoptimal temperature range in response to the different temperatures for MR73 cultivated rice variety, Seberang Perak, Kuala Pilah, KADA, Besut and Perlis weedy rice strains. T_b, T_o, and T_c indicate base, optimum and maximum temperature, respectively. * and **, significant at P<0.05 and <0.01, respectively*

The higher germination percentage in the cultivated variety at different constant temperatures (10-30°C) could be attributed to the relatively higher germination rate (Table 3). Similarly, a lower germination percentage in weedy rice strains was due to the lower germination rate, particularly at 25°C and lower. The highest germination rate in the cultivated variety and the weedy strains was observed at 25 °C and 30 °C, respectively.

Germination Rate and Cardinal Temperatures Based on the Linear Model

The estimated germination rates, within the sub-optimal and supra-optimal range of temperatures, vary between the weedy rice strains. All the germination rates, which were calculated from the estimated germination time course, showed a significant correlation with temperature at both the sub-optimal and supra-optimal ranges of temperatures (*Fig. 1*). The highest estimated germination rate was recorded for MR73, which was 0.226 day^{-1} in the sub-optimal range. On the contrary, the lowest estimated germination rate

was observed in the KADA strain (0.014 day^{-1}), while the highest was in SP strain with 0.128 day^{-1}, based on the linear regression model in the sub-optimal range.

The decline in the germination rate within the supra-optimal range for the weedy rice strains was between -0.078 day^{-1} to -0.33 day^{-1}. Meanwhile, the cultivated rice variety and SP weedy strains had similar germination rate (within the supra-optimal range of temperature), suggesting that the weedy rice strain has a similar germination characteristic with the cultivated rice variety (MR73) at higher temperature. Within this supra-optimal range of temperature, the KADA weedy strain was found to have the lowest estimated germination rate of -0.078 day^{-1}.

The germination rate for the weedy rice strains and the cultivated variety increased linearly with the increase in the germination temperature (*Fig. 1*). Meanwhile, the lowest estimated T_b for MR73 was 0.4°C (Table 4 and *Fig. 1*). The range of the estimated T_b for the weedy rice strains was between 2.0 - 7.3°C, while the KP strain had the lowest T_b. The T_o for the

TABLE 3
The germination rate at different imbibition temperatures of a cultivated rice variety and
weedy rice strains

Variety/strain	Germination rate (d^{-1})					
	10°C	15°C	20°C	25°C	30°C	35°C
MR 73[†]	2.9	3.9	4.7	4.9	4.7	1.5
Seberang Perak	0.4	0.5	2.7	3.1	4.1	2.7
Kuala Pilah	1.0	1.6	2.5	2.7	3.0	3.0
KADA	0	0	0	0.4	1.1	0.6
Besut	0	0	1.7	1.4	2.4	1.7
Perlis	0.3	0.4	1.0	1.2	2.5	2.8
LSD	1.3	0.8	0.9	0.7	1.1	1.0

[†] cultivated rice variety

seed germination ranged from 28.1 to 37.5°C for the weedy rice strains (Table 4). The estimated T_o for MR73 was found to be the lowest (24.3°C) as compared to the weedy rice strains, whereas the highest T_o of 37.5°C was observed in the KADA strain. Nonetheless, T_c did not differ much between the weedy rice strains and MR73. The narrow range of T_c among the two varieties was between 42.2 – 43.3°C, suggesting that the non-dormant rice seed will not germinate above 43°C.

DISCUSSION

Under ideal germination environments, such as in the laboratory condition, seed dormancy is strongly imposed in some weedy rice strains. Weedy rice strains have often been associated with seed dormancy (Gu *et al.*, 2005). The results indicated that the seed of the KADA strain is highly dormant relative to the seeds of other strains. However, the data presented in this study are still insufficient to determine the type or class of dormancy involved in the tested weedy rice strains. Reducing the percentage of viability between the TTC test and the standard germination test will indicate the degree of dormancy of a seed lot. The SP strain does not have seed dormancy, suggesting that not all weedy rice strains have seed dormancy. It appears that the strain of SP is closely related to the cultivated variety. Therefore, those weedy rice strains producing non-dormant seeds will result in a more widespread infestation in the field throughout the year.

The degree of seed dormancy in weedy rice varies between the strains. Since the strains used in this study were collected from different locations, environmental conditions are therefore suggested to play important roles in determining the degree of dormancy. The variation in the degree of seed dormancy is not only influenced by the environment at the location where the plants are grown, but it is also influenced by genetic factors (Li and Foley, 1997; Gu *et al.*, 2005). The rate of germination appears to be related to the degree of dormancy, but it does not seem to be related to the range of cardinal temperatures. The seed of the KADA strain was found to be very dormant and thus had the lowest germination rate. However, the range of the cardinal temperatures, T_b to T_c, was quite similar to the non-dormant seed of the SP strain. The results indicated that for the non-dormant seed (e.g. in cultivated MR73 variety), T_b would be shifted to a much lower temperature relative to the weedy strains. Meanwhile, T_o for the weedy strains, except for the KP strain, was above 30°C. In this study, the increase in the degree of seed dormancy does not shift T_c in rice, but it changes T_o to a much higher temperature.

T_c was almost similar among the weedy rice strains and the cultivated variety. This suggests that the maximum temperature limit for seed germination is species specific, and it is not influenced by seed dormancy. It is interesting to note that the degree of seed dormancy will change, i.e. either shorten or widen, the sub-optimal and supra-optimal ranges of temperature. This study has clearly indicated that the sub-optimal range of the temperature of the dormant seed is widened by 26-31°C temperature points compared to the non-dormant cultivated variety and the SP strain. The supra-optimal range of temperature is concurrently shortened with the increase in seed dormancy. The results also provide evidence that there is an ecotypic variation in the base and optimum temperatures for seed germination in weedy rice.

Fluctuation in soil temperatures is commonly associated with weed seed emergence in several species (Foncella *et al.*, 2000; Vleeshouwers and Kropff, 2000). However, in tropical growing environments, fluctuation in soil temperature may be negligible, yet higher weedy rice infestation is commonly observed when temperature during preceding growing season is higher. Higher T_o requirement for the weedy rice strains observed in this study could possibly trigger the germination process and the reason for high weedy rice infestation when the temperature during preceding growing season was above normal.

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Characteristics of Pulp Produced from Refiner Mechanical Pulping of Tropical Bamboo (*Gigantochloa scortechinii*)

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ABSTRACT

Bamboo properties are somewhat similar to certain timbers but it has an advantage of having longer fibres, making it suitable for the production of pulp for paper and hardboard. However, the pulping process is a very crucial stage to produce fibres with an optimum quality. This study was carried out to characterize the pulp of *Gigantochloa scortechinii* using refiner mechanical pulping (RMP). The parameters evaluated included the effects of pre-treatment soaking in NaOH or steaming of chips and effects of refiner plate gap on pulp quality. Pulp quality was assessed based on the properties, yield, and lignin content of fibres. The pre-treatment with NaOH at 60°C for 6 h was found to produce superior quality pulp and lesser lignin content compared to pre-treatment by steaming at 150°C for 3 h. Meanwhile, the refiner plate gap test showed that the two cycles of refining (2.5-mm followed by 0.5-mm plate gap) reduced the lumpiness of the fibre, but it had lower felting power and Runkel ratio. Two cycles of refining process also led to higher fibre yield, produced more unbroken and slender fibres as compared to when one cycle treatment using 2.5-mm plate gap was used.

Keywords: ***Gigantochloa scortechinii*, refiner mechanical pulping, bamboo pulp**

INTRODUCTION

Bamboo has gained a great attention as potential raw material for wood-based industry in Malaysia. The bamboo plant can be harvested from its natural habitat or grown in a large scale (Azmy and Abd. Razak, 2000). *Gigantochloa scortechinii*, which is locally known as *Buluh semantan* is one of the most common species harvested and its use is mostly associated with traditional uses. Today, bamboo has been explored and expanded for high value-added products such as composites and laminated products. For a number of years, work has been carried out in bamboo producing countries to enhance the utilisation and range of products that can be manufactured from bamboo (Ganapathy, 1999).

Bamboo has the properties which are somewhat similar to certain timbers (Azmy and Abd. Razak, 2000), but it has an advantage of having longer fibres which makes it suitable for the production of pulp for paper and hardboard. However, bamboo is very hard compared to wood (Ganapathy, 1999) and its pulping process, especially when done mechanically, will impose problems if the material is not initially softened. A pre-treatment of the material is thus required prior to refiner mechanical pulping to obtain smooth and unbroken fibres for making hardboard (Kollmann *et al.*, 1975). One of the

common pre-treatments is the conventional steaming in which higher temperature and pressure are used to soften fibres. Nonetheless, this process will produce more brittles and higher amount of broken fibres after mechanical refining.

Another potential method is through mercerisation using sodium hydroxide solution. The alkali solution helps to degrade lignin and soften fibre physically and chemically (Sreekala *et al.*, 1997). In particular, sodium hydroxide treatment functions as an irreversible mercerization effect to increase amorphous cellulose. This process is vitally important to yield high fibre recovery after refining and prevent fibre breakage or damage. In refiner mechanical pulping, besides pre-treatment process, the quality of pulp is also influenced by the gap of refiner disc plate. A high refiner plate gap will only produce loose fibres and the tendency to become lumpy is also greater, while smaller plate gap will lead to finer fibres, and hence reduce the yield.

This paper discusses the characteristics of pulp yielded from the pre-treatment of tropical bamboo (*Gigantochloa scortechinii*), either by steaming or by soaking in NaOH solution prior to refiner mechanical pulping (RMP). RMP was chosen in this study because it incurred lower cost as compared to chemical pulping and also prevented bamboo fibres from producing fines that could decrease the yield (Rowell *et al.*, 2000) as well as hardboard strength (Beg and Pickering, 2004). The effects of refiner plate gap and the number of refining cycles on the properties of fibres are also reported.

MATERIALS AND METHODS

Materials

Fresh bamboo culms of *Gigantochloa scortechinii* Gamble (around 3-4 years old) were obtained from the Forest Research Institute Malaysia (FRIM) Research Plot at Chebar Besar Forest Reserve of Nami, in Kedah, Malaysia. A bamboo splitter that has eight fractions was used to split each culm into eight splits. The epidermis

and nodal parts of the splits were removed using a single-faced planner. The bamboo strips produced had an approximate dimension of 20-mm (width), 4-mm (thickness), and 1000-mm (length), chipped to approximately 20 mm x 20 mm pieces and air-dried until equilibrium with the surrounding moisture content (MC). The air-dry MC of the chips was determined using the standard oven-drying method.

Mechanical Pulping of Bamboo Chips

The chips were divided into two batches. The first batch was steamed in a digester at 150°C with a pressure maintained at 5.95 kgcm^{-2} for 3 h. For this process (during the first 1 h period), the temperature and pressure were gradually increased, while the final temperature and pressure were maintained for the next 2 h. The second batch was soaked in 2% NaOH solution and maintained at 60°C (Sreekala *et al.*, 2002) for 4, 6 or 8 h. The optimum soaking time for this treatment was evaluated. This is very important to prevent the fibre from being over-treated which could reduce its properties (Beg and Pickering, 2004). After soaking, the chips were washed thoroughly with cold water to remove sugars which could affect refining of chips (Rowell *et al.*, 2000).

The pre-treated chips were mechanically defibrated using a single disc refiner (Andritz Sprout-Bauer Model). The effects of the plate gaps and the number of refining cycles on the properties of fibre were also investigated. Three stages of refining were conducted with the disc plate gaps set between the refining plates at 2.5 mm, 0.5 mm and 0.1 mm, respectively. The preliminary results showed that with the use of 2.5 mm plate gap, the fibre yield recovery was approximately 60%, whereas those produced after refining at 0.1 mm plate gap size had only 40% fibre yield recovery. Thus, only the fibre morphology of the refined fibres, using 2.5 mm and 0.5 mm plate gaps, was analyzed. The refining process was performed in two different cycles; first with a refiner plate gap of 2.5 mm only, and secondly, initial refining using 2.5 mm, followed by 0.5 mm plate gaps. The purpose of

the two-cycle refining was to reduce the adverse effect of harsh actions by the plates to the fibres. After refining, the wet fibres were manually squeezed to get rid of the water. Fibres from the untreated bamboo were macerated according to the standard laboratory manual and the data were used for comparison purposes.

Fibre Evaluation

Fibre yield recovery was determined by calculating the mean oven dry weight of the fibres yielded from the refiner per kilogramme of chips input. One g each of the wet fibres from different pre-treatments was stained in safranin 1%. They were then washed in alcohol series of 30%, 50%, 70%, and 95% alcohol each for 2 min, and finally with xylene. Several strands of fibres were placed on the slide, covered with a glass cover and labelled. The observations were made on a Leitz DMRB Image Analyzer

which was attached to a digital camera. The length, diameter, and cell wall thickness of the fibres were determined by direct measurement of the magnified image of the fibres mounted on the slide. Fifty measurements were made from each of the five slides. Photographs of the sections (i.e. 20x magnification) were taken and printed.

The colour of the pulp was examined by comparing the pulp solution (10% w/v) from each process with Munsell Soil Colour Charts. Meanwhile, the lignin content of the pulp, together with the untreated bamboo, was determined according to the TAPPI Standard T222 OS-74 (Anonymous, 1974).

The analysis of variance (ANOVA) was performed on fibre property values to detect any differences between the pre-treatment processes and the numbers of refining cycles.

TABLE 1
Fibre properties of *G. scortechinii* yielded from different pre-treatments of chips
followed by 2 cycles of RMP

Fibre characteristics	Pre-treatments				
	Steaming at 150°C and 5.95 kgcm^{-2}	Soaking in 2% NaOH at 60°C			Laboratory processed fiber
		4 h	6 h	8 h	
Fibre yield (%)	50.7c	65.2b	77.2a	74.6a	-
Length, *L* (mm)	1.45d	1.66c	1.96b	1.71c	3.20a
Width, *D* (μm)	26.84a	26.77a	26.77a	26.55a	20.64b
Cell wall thickness, *w* (μm)	5.51c	10.32a	10.76a	10.74a	6.90b
Lumen width, *l* (μm)	17.82a	6.14c	5.25d	5.07d	14.04b
Felting power (*L/D*)	54	62	73	64	156
Runkel ratio (2*w/l*)	0.62	3.36	4.10	4.24	4.17
Lignin content (%)	25.67a		19.90b		26.94a
Colour of fibre solution	Dark brown		Yellow		Pale yellow

Means followed by the same letter are not significantly different at p < 0.05 using LSD

RESULTS AND DISCUSSION

The results for the fibrous properties of *G. scortechinii* from different pre-treatment processes, followed by 2 cycles (2.5-mm plate gap followed by 0.5-mm plate gap) of the mechanical pulping, are shown in Table 1. The properties of fibres resulted from refining using different plate gaps are given in Table 2.

Effect of Pre-treatments on Fibre Properties

In this study, the fibre recovery from the steam pre-treatment of bamboo (50.7%) was lower than NaOH pre-treatment (65.2- 77.2%). In particular, soaking in NaOH for 6 h yielded the highest recovery. A higher percentage of broken fibres was found in the steam-treated bamboo and the fibre produced was rather rigid (*Fig. 1a*). This was probably attributed to the lignin which was still present in a large quantity (25.67%) in the loose fibre. The lignin content in the untreated bamboo was found to be around 26.94%, while the lignin content in NaOH-treated fibre was 19.9% (Table 1). The results reflected that the steaming temperature used in this study (150°C) was insufficient to degrade the lignin from the fibre (Suchsland and Woodson, 1991; Sjostrom, 1993). Both the thermal and mechanical actions involved in the pulping

process could also lead to the brittleness of the fibre, causing it to collapse easily and form fines. Steaming process has also been found to produce lumpy fibres. This is a result of flocculation where water content in the fibres is relatively higher, and thus making them more absorbent or hydrophilic. Unlike the steam pre-treated fibre, fibres which were pre-treated with NaOH were hydrophobic. The treatment had partially removed lignin and hence produced microfibril with greater crystallinity.

The high fibre recovery from the NaOH pre-treatment is partly due to the effectiveness of this particular process in changing cellulose I to cellulose II, resulting in increment of crystalline fibre chain and reduction of amorphous line. Natural cellulose has cellulose I crystalline structure, but on alkalisation, it changes to cellulose II, in which the parallel polymer chains of cellulose I was aligned anti-parallel and higher exposition of OH⁻ (Vilaseca *et al*., 2006). The high content of hemicellulose, coupled with the reduction of lignin during alkalisation, would contribute to more fibres being easily extracted from the treated chips. Pickering *et al*. (2006) found that chemically processed fibre was 32% richer in cellulose as compared to the non-chemically processed fibres. NaOH pre-treatment helps swelling the fibrils and also

TABLE 2
Fibre properties of *G. scortechinii* chips produced after refining at different cycles

Fibre morphology	Refiner plate gap size (mm)			
	Steaming at 150°C and 5.95 kgcm⁻²		Soaking in 2% NaOH at 60°C for 6 h	
	1 cycle	2 cycles	1 cycle	2 cycles
Length, L (mm)	2.39a	1.45b	2.18a	1.96b
Width, D (micron)	26.58a	26.84a	26.63a	26.77a
Cell wall thickness, w (μm)	4.80b	5.51b	10.72a	10.76a
Lumen width, l (μm)	18.98b	17.82b	5.19a	5.25a
Felting power (L/D)	90	54	82	73
Runkel ratio ($2w/l$)	0.51	0.62	4.13	4.10

Means within the pre-treatment followed by the same letter are not significantly different at p < 0.05 using LSD

cleaning the fibre bundle surface (*Fig. 1b* and *c*), and as a result increased the tensile strength of the fibre. As the fibre strength improved and became more plastic, it would not be easily damaged by mechanical pulping and more fibre could be extracted (Pickering *et al*., 2006; Mwaikambo and Ansell, 2003).

The results also showed that the quality of fibre from NaOH pre-treatment was dependent on the soaking time. The longest fibre was recorded from 6 h soaking (1.96 mm) and the shortest was in 4 h (1.66 mm). Nonetheless, prolonging the soaking time to 8 h did not significantly affect the length of fibre (1.71 mm). The steam treatment was found to produce shorter fibre (1.45 mm) as compared to the NaOH treatment. The high variation of fibre length in the steam treatment (71.1%) indicated that the fibres were broken during pulping (*Fig. 1a*), as a result of embrittlement of the fibres caused by the application of high temperature (150°C) and pressure (5.95 kgcm^{-2}). Generally, the mechanical pulp had fibres shorter than

the actual fibres produced using the laboratory mercerisation process (i.e. 3.20 mm).

Regardless of the pre-treatment process, the width of fibre produced by RMP was relatively similar, i.e. between 26.55-26.84 µm. These values were relatively higher than the fibres extracted in the laboratory. Similarly, the thickness of the cell wall for the NaOH-treated fibre (10.32-10.74 µm) was significantly higher than that of the fibre which was produced in the laboratory (6.90 µm). A similar observation was reported by Mwaikambo and Ansell (2003). The NaOH treatment was found to help the cell wall to swollen and to produce fibre with small lumen size known as closed lumen (*Figs. 1b* and *1c*). An internal fibrillation of the cell wall was also noticed in the NaOH-treated fibres (*Fig. 1d*). Lignocellulosic fibre is usually packed with microfibrils but it was split after the alkali treatment (Cao *et al*., 2006). This phenomenon is termed as fibrillation that breaks the treated fibre bundle down into smaller ones by the dissolution of the hemicellulose. Fibrillation
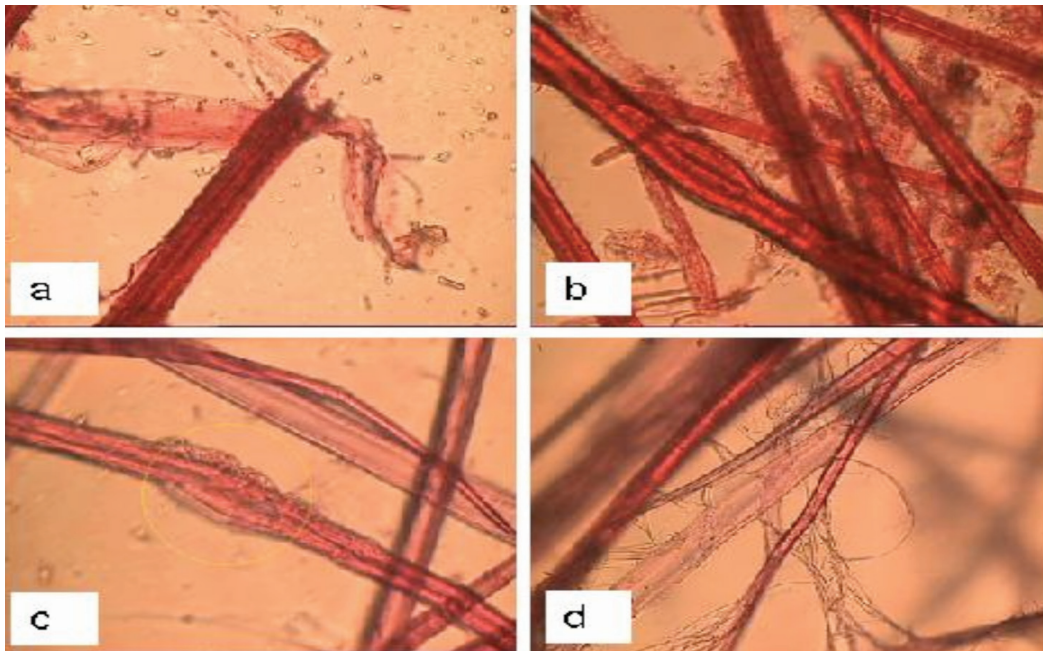

Fig. 1 (a): Broken fibre from steam-treated chips, (b) Swollen unclean surface fibre from 4 h-NaOH-treated chips, (c) Swollen clean surface fibre from 8 h-NaOH chips, and (d) Fibrillation of fibres from NaOH-treated chips

has the advantage of increasing the surface area available for contact with the matrix and hence improving the interfacial adhesion (Bisanda and Ansell, 1992).

Higher felting power results in a better fibre-matrix adhesion (Gassan and Bledzki, 1999; Mwaikambo and Ansell, 2003; Cao *et al*., 2006). Fibre with high felting power indicated that the fibres produced are very slender (long and thin fibres) and with good pulp quality (Britt, 1970). The felting power of the steam-treated fibres was 54 and this was 73 for the NaOH-treated fibre. The values are very much lower as compared to the laboratory produced fibre (146), but it is similar with the range values for the southern pine (28-440) (Sjostrom, 1993). The results also suggested that the felting power for the mechanical pulping bamboo fibre would give a good interfibre bonding in the production of paper or hardboard. The NaOH-treated fibres had high Runkel ratio (i.e. with 4.10), while steam-treated fibres had 0.62. Meanwhile, the Runkel ratio of the untreated fibres was 4.17.

As for colour, the solution of steam-treated fibre is darker (dark brown) than NaOH-treated fibre (yellow), while the solution of the untreated fibre has a yellowish colour when the Munsel Soil Colour Chart was used as a reference. The darker colour found in the steam-treated fibre is partly due to the heat and pressurised system in the process which decolourises the fibres, and this may probably be due to the high amount of lignin retained in the fibres (25.67%). Fengel and Shao (1985) reported that lignin softens and becomes thermoplastic at 90°C, while degrades and dissolves when the temperature reaches 170°C. However, the temperature used in this study was only 150°C which maintained the lignin in a plastic form in the fibre structure (Hsu *et al*., 1986). In this study, the NaOH treatment was found to remove only 7.04% lignin from the bamboo. Therefore, the low concentration of NaOH solution (2%) used in this study might not be sufficient enough to degrade all the lignin. Nonetheless, the treatment with 5% NaOH successfully removed a great amount of lignin from palm fibres (Geethamma *et al.*, 1995),

while the treatment with 6% NaOH removed lignin of hemp, jute, sisal, and kapok fibres (Mwaikambo and Ansell, 2003).

Effect of Refiner Plate Gap on the Properties of Fibre

Adjustment of refiner plate gap would vary the size of the fibre bundles of wood (Blomquist *et al*., 1981). The effects of refiner plate gap on the fibre properties are shown in Table 2. In general, chips which underwent one cycle of refining had a higher fibre length than those refined for two cycles, regardless of the pre-treatment. The pulps produced, however, were lumpy (*Fig. 2*). For the steam-treated chips, the length of fibre was reduced from 2.39 mm to 1.45 mm, when they were refined from one cycle to another. Those treated with NaOH for 6 h produced fibre with the mean lengths of 2.18 mm and 1.96 mm when refined for one and two cycles, respectively. Wood fibre has been reported to be easily damaged by the rotating knife when it was first treated with steam (Das *et al*., 2000).

The diameters of fibre from both the pre-treatments were not significantly different from each other. The values ranged from 26.22 to 26.84 μm. Meanwhile, the thickness of the cell wall of the NaOH-treated fibre which underwent one cycle and two cycles of refining was similar (10.72-10.76 μm). However, steam-treated fibres with two cycles of refining had a thicker wall (5.51 μm) as compared to a single cycle of refining (4.80 μm). The increment in the thickness of the cell wall after the second refining was attributed to the flattening or collapse of fibre due to the narrow gap of plate action. Clark (1985) revealed that the high temperature steaming, coupled with the harsh action of refiner plate gap, would easily cause fibre to collapse. Regardless of the pre-treatment, the felting power of the fibre was adversely affected by the number of refining cycles. A higher felting power (90) was found on the steam-treated fibre which had undergone 1 cycle of refining. It was decreased to 54 when refined with a smaller plate gap. The same result was

*Fig. 2. Fibres from two cycles (2.5-mm plate gap followed by 0.5-mm) of refining (left)
and Lumpy fibres from one cycle (0.5-mm plate gap) of refining*

observed for the NaOH-treated fibres, where two cycles of refining produced a higher felting power fibre (82) than that of one cycle (73), but with insignificant variation as compared to the steam-treated fibre. The Runkel ratios of the NaOH-treated fibre, with one and two cycles of refining, are similar (i.e. between 4.10-4.13) and these values were markedly higher than the steam-treated fibre where the values are 0.51 and 0.62 for one and two cycles, respectively.

to the one-cycle refining (2.5 mm plate gap). This treatment also resulted in higher fibre yield, produced more unbroken and more slender fibres than the one-cycle treatment. The results also revealed that an optimum quality of mechanical bamboo pulps for hardboard production could be obtained through pre-treatment of chips by soaking in 2% NaOH for 6 h, followed by 2 cycles of refining (first with 2.5-mm and followed by 0.5-mm plate gaps).

CONCLUSIONS

The pre-treatment of *G. scortechinii*, prior to mechanical pulping, was found to significantly affect the pulp properties. The results showed that soaking bamboo in 2% NaOH solution maintained at 60°C produced higher fibre recovery, superior quality, and lighter pulp colour than those which were pre-treated by steaming at 150°C for 3 h. Within the NaOH treatments, bamboo soaked for 6 h produced an optimum pulp quality compared to 4 and 8 h soakings. In the refiner plate gap test, two cycles of refining (2.5 mm followed by 0.5 mm plate gap) reduced the lumpiness of the fibre, but it had a lower felting power and Runkel ratio compared

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