CHAPTER 4: RESULTS

4.1 DESCRIPTION OF *GRACILARIA* SPECIES

The alga samples collected were separated into three species: *Gracilaria changii* (Xia et Abbott) Abbott, Zhang et Xia, *Gracilaria salicornia* (C. Agardh) Dawson and *Gracilaria edulis* (Gmelin) Silva according to the description by Phang, 1994. Photographs of *G.changii*, *G.salicornia* and *G.edulis* collected from Morib, Malaysia are shown in Figures 7-9.

4.1.1 *Gracilaria changii* (Xia et Abbott) Abbott, Zhang et Xia

Basionym : *Polycavernosa changii* Xia et Abbott

Synonym : *Hydropuntia changii* (Xia et Abbott) Wynne

Description: Generally, plants are bushy, purplish brown to dark brown when dry. The main branches are much thicker compared to the third order branches. Thallus is turgid, cylindrical, abruptly constricted at the base forming a slender stipe, slightly swollen distal of stipe, tapering towards the tip.

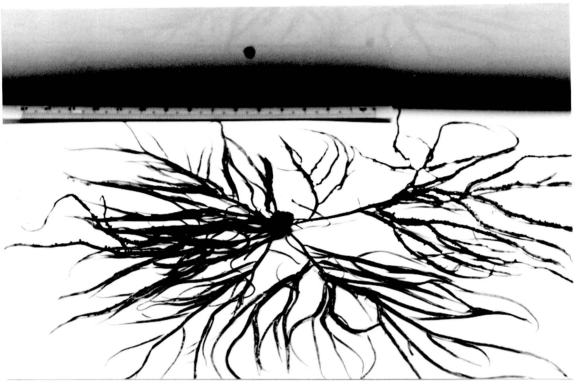


Figure 7: Photograph showing species of *Gracilaria changii* collected from Morib

4.1.2 *Gracilaria salicornia* (C. Agardh) Dawson

Basionym: *Sphaerococcus salicornia* C. Agardh

Synonyms: *Corallopsis salicornia* (C. Agardh) Greville, *Corallopsis dichotoma* Ruprecht, *Corallopsis cacalia* J. Agardh, *Corallopsis minor* (Sonder) J. Agardh, *Fucus salicornia* Mertens mscr., *Gracilaria crassa* Harvey, *Gracilaria cacalia* (J.Agardh) Dawson, *Gracilaria minor* (Sonder) Durairatnam, *Gracilaria canaliculata* (Kutz.) Sonder

Description: The main feature in distinguishing this species is the constricted branches, which form obcuneate articulations. Irregular to dichotomous, trichotomous or divaricate branching. Plants with regular branching that forms tightly entangled masses when internodes are long but low prostrate clumps when internodes are short. Thallus is cylindrical, brittle when fresh, light orange to dark purple or greenish in colour. Plants grow in silted muddy mangroves are found to have long internodes. Short prominently clavate internodes are associated with those found on rock and corals in intertidal areas.

Figure 8: Photograph showing species of *Gracilaria salicornia* collected from Morib

4.1.3 *Gracilaria edulis* (Gmelin) Silva

Basionym : *Fucus edulis* Gmelin

Synonyms: *Polycavernosa fastigiata* Chang et Xia

Hydropuntia fastigiata (Chang et Xia) Wynne

Description: Plants arise from discoid holdfast, up to 27cm tall with dichotomous or trichotomous branches, which may have or may not have slight constriction at bases. Thallus is cylindrical and ends in pointed apices. Plants are much branched, wiry and entangled with either short or spinelike ultimate branches or with tendrils. Plants found attached to fish cages have thicker primary branches (1.5mm) compared to those growing in mangroves (0.5-1.0mm). However, plants found on corals are bushy with short branches that are short, fine, almost spinelike ultimate branches.

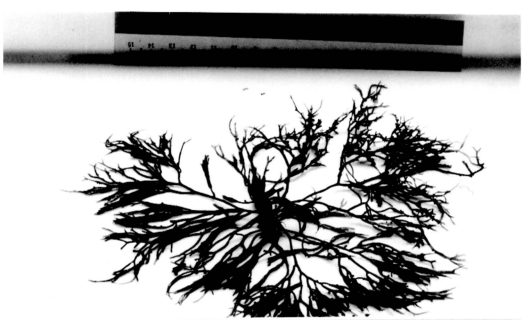


Figure 9: Photograph showing species of *Gracilaria edulis* collected from Morib

Gracilaria changii was collected from six geographical locations. Figures 10-15 show the photographs of *Gracilaria changii* collected from these locations. Although all samples were identified as *Gracilaria changii*, some morphological differences were observed (Table 4).

Figure 10: Photograph showing *Gracilaria changii* from Phuket, Thailand

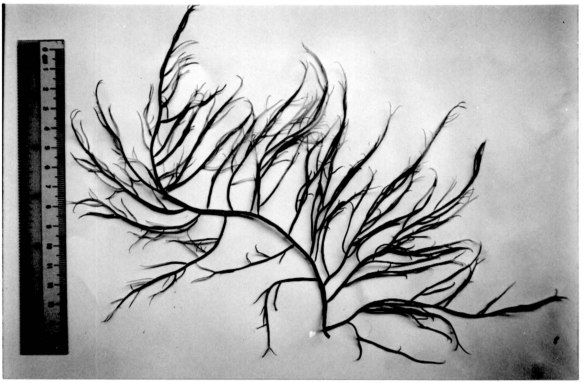


Figure 11: Photograph showing *Gracilaria changii* from Ban Merbok, Malaysia

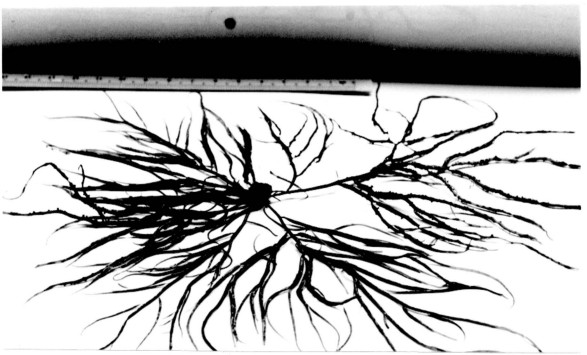


Figure 12: Photograph showing *Gracilaria changii* from Morib, Malaysia

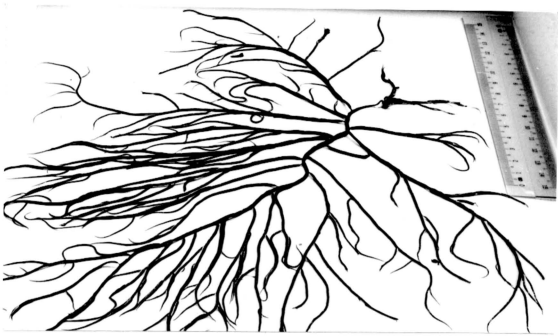


Figure 13: Photograph showing *Gracilaria changii* from Carey Island, Malaysia

Figure 14: Photograph showing *Gracilaria changii* from Sungai Pulai, Malaysia

Figure 15: Photograph showing *Gracilaria changii* from Takbai, Thailand

(Herbarium Material)

Table 4 : Description of the *Gracilaria changii* collected from different locations

Locations	Description			Reproductive stage
	Appearance of thallus	Thallus diameter	colour	
Phuket, Thailand	Thallus are robust	0.54-1.05 mm	slightly greenish brown	Generally non-reproductive, occasionally few cystocarpic plants
Takbai Thailand	Highly proliferating and fine, long thallus	0.35-1.00 mm	yellowish brown	non-reproductive
Ban Merbok	Highly proliferating and fine, long thallus	0.38-1.00 mm	light brownish red	non-reproductive
Morib	Thallus more robust than above	0.53-1.76 mm	slightly yellowish brown	Generally reproductive, high frequency of cystocarpic plants
Carey Island	Thallus very robust and healthy looking	0.69-1.84 mm	dark brownish red	Generally non-reproductive, occasionally few cystocarpic plants
Sungai Pulai	Thallus are robust	0.55-1.17 mm	dark brownish red	Generally non-reproductive, occasionally few cystocarpic plants

4.2 DEOXYRIBUNUCLEIC ACID (DNA) EXRACTION AND ISOLATION

DNA from selected *Gracilaria* species was isolated using two protocols. Protocol 1 was adapted from Sinnappah (1994) while protocol 2 was a modification of Protocol 1. DNA yield, DNA concentration and $OD_{260/280}$ ratio obtained from the isolation of DNA from *Gracilaria* samples using both protocol 1 and 2 are given in Table 5. DNA isolated using protocol 2 gave higher $OD_{260/280}$ and DNA yield compared to DNA isolated using protocol 1. Figure 16 shows the agarose gel of genomic DNA isolated from *Gracilaria changii* using protocol 2. Most of the DNA isolated from the *Gracilaria* samples using this protocol 2 gave polymorphic band patterns in RAPD.

Table 5: Comparison of the purity and quantity of DNA obtained from *Gracilaria* samples isolated using Protocol 1 and Protocol 2.

DNA extraction method	$OD_{260/280}$	DNA concentration	DNA Yield
Protocol 1	1.000-1.570	10-17 μg/ml	3.33μg/g-5.67μg/g
Protocol 2	1.043-2.452	50-528μg/ml	16μg/g-176μg/g

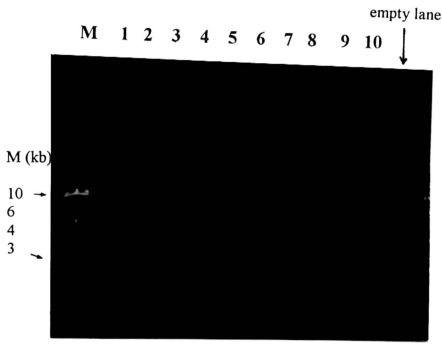


Figure 16: Genomic DNA isolated from representatives of *Gracilaria changii* using Protocol 2 on 0.8% agarose gel, M, High DNA mass ladder.

Protocol 2 was used to isolate DNA from all the *Gracilaria* samples collected from different locations. Table 6 shows the quality and quantity of DNA isolated using this protocol. The purity of DNAs obtained ranged from 1.043-2.452 while the DNA yields obtained ranged from 50-528μg/ml. The values were in a wide range and they were not constant for all the samples of *Gracilaria*. However, most of the DNA samples isolated using this protocol gave polymorphic DNA band pattern of RAPD despite the low purity.

Table 6: The quality and quantity of DNA isolated from *Gracilaria changii* collected from different locations using protocol 2

Collection sites	No. of samples	OD260/280	DNA concentration
Carey Island	31	1.311-2.116	50-475 μg/ml
Morib	30	1.239-2.452	50-278 μg/ml
Ban Merbok	19	1.043-1.232	50-528 μg/ml
Sungai Pulai	34	1.108-1.366	50-368 μg/ml
Phuket, Thailand	4	1.088-1.123	100-399 μg/ml
Takbai, Thailand	4	1.096-1.154	100-523 μg/ml

4.3 RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD)

4.3.1 Screening of Primers and Optimisation of PCR reaction

The 20 primers of Operon Kit A (Operon Technologies, California, USA) were screened for suitable primer to be used in the RAPD analysis (Table 3). RAPD reaction was carried out with the 20 primers on the same DNA template isolated from *Gracilaria changii* (Morib). Of these primers, four primers (OPA3, OPA10, OPA11, OPA13) were able to generate polymorphic amplification product (figures 17-20). RAPD was carried out at four different annealing temperatures: 33°C (figure 17), 36°C (figure 18), 38°C (figure 19) and 40°C (figure 20) for the four primers. The negative control consists of PCR mixture without the presence of template DNA. No band was found in the lanes contain the negative control. However, the bands patterns generated for the same combination of primer and DNA template under different temperature were similar but varied in the number of bands amplified. High temperature (38°C and 40°C) gave more bands compared to lower annealing temperature (33°C and 36°C). Annealing temperature of 38°C was used consistently for the later experiments.

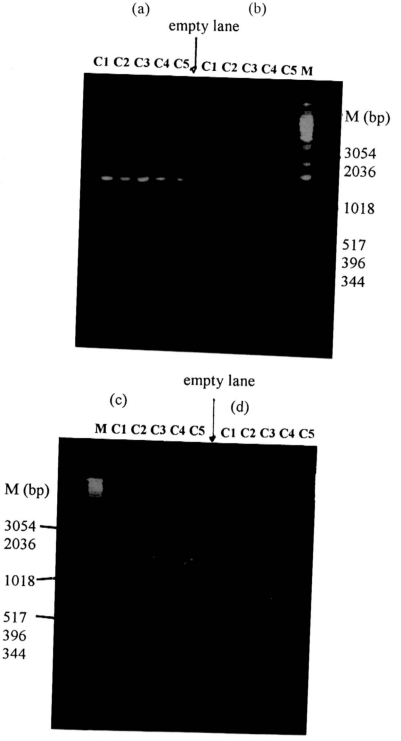
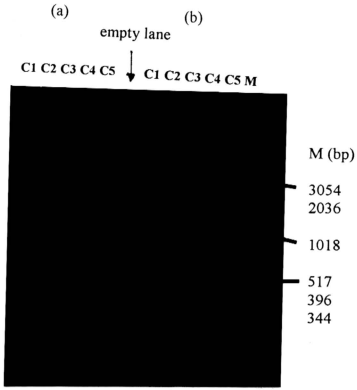


Figure 17: RAPD bands patterns generated for the different DNA templates of *Gracilaria changii* (C1, C2, C3, C4, C5) with primer OPA3 (a), OPA10 (b), OPA11 (c) and OPA13 (d) at 33°C. M, 1kb DNA ladder.

(No band was found in the lane consist of negative control—result not showed)



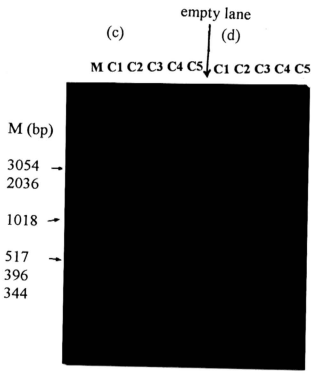


Figure 18: RAPD bands patterns generated for the different DNA templates of *Gracilaria changii* (C1, C2, C3, C4, C5) with primer OPA3 (a), OPA10 (b), OPA11 (c) and OPA13 (d) at 36°C. M, 1 kb DNA ladder.

(No band was found in the lane consist of negative control—result not showed)

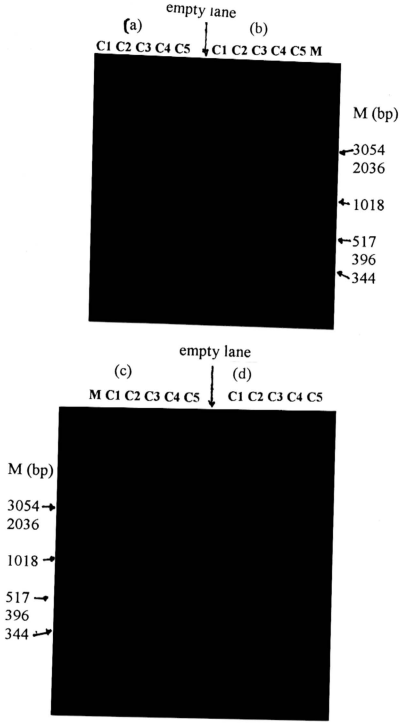


Figure 19: RAPD bands patterns generated for the different DNA templates of *Gracilaria changii* (C1, C2, Ç3, C4, C5) with primer OPA3 (a), OPA10 (b), OPA11 (c) and OPA13 (d) at 38°C. M, 1 kb DNA ladder.
(No band was found in the lane consist of negative control—result not showed)

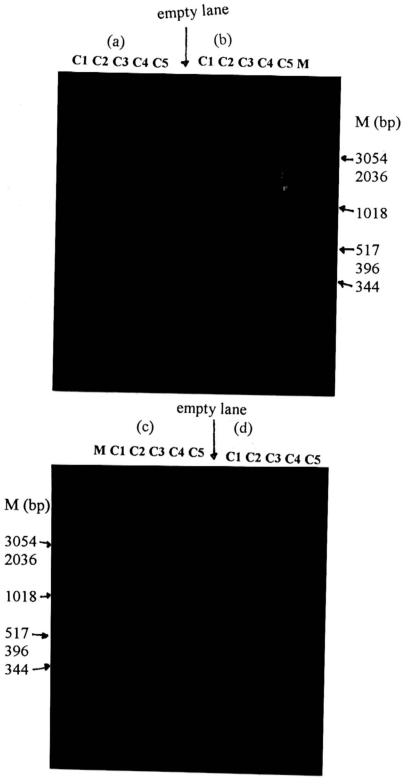


Figure 20: RAPD bands patterns generated for the different DNA templates of *Gracilaria changii* (C1, C2, C3, C4, C5) with primer OPA3 (a), OPA10 (b), OPA11 (c) and OPA13 (d) at 40°C. M, 1 kb DNA ladder.

(No band was found in the lane consist of negative control—result not showed)

4.3.2 Reproducibility

To assess the reproducibility of RAPD, amplifications were performed on different occasion on identical DNA samples from *Gracilaria changii* collected from Morib using OPA10. Generally, the RAPD generated band patterns show good reproducibility although some variability was noted (Figure 21). Major bands were observed to be constant with all the amplifications but minor bands were more variable. Although, lane 5 (Figure 21) showed a slight difference compared to other lanes (probably due to contamination during the PCR mixture preparation), the overall result showed that RAPD gave good reproducible bands pattern.

Figure 21: Assessment of reproducibility of RAPD. RAPD pattern generated using DNA from *Gracilaria changii* from Morib with OPA10. M, 1 kb plus DNA ladder.

4.3.3 RAPD Profiles

Of 20 primers of Operon Kit A (Operon Technologies, California, USA) screened, four primers (OPA3, OPA10, OPA11 and OPA13) were chosen to produce the RAPD profiles of all the samples for comparison. Different primers gave different polymorphism with various numbers of bands with different samples (Figures 22-41). However, less than 20 bands were generated for each primer-DNA combination. Generally, the size of amplified DNA fragments generated by the four primers for all the samples tested ranged from 200bp to 3000bp.

4.3.4 RAPD Profiles Using Primers OPA3, OPA10, OPA11 and OPA13

There were some common bands and specific bands found among the samples using the four selected primers (OPA3, OPA10, OPA11 and OPA13). For example, primer OPA3 gave a common band of 629.43bp among the *Gracilaria changii* collected from Malaysia (Figures 22-25). Common bands were also found specific to one population such as 1244bp and 1660bp for Carey Island samples (Figure 22) while 1207bp and 1443bp were common to the Morib samples with OPA3 (Figure 23).

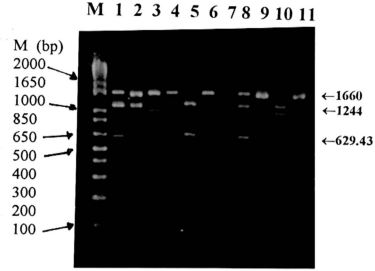


Figure 22: RAPD profile for different DNA samples isolated from *Gracilaria changii* of Carey Island (N) with OPA3 (Lanes 1-11). M, 1 kb plus DNA ladder. (No band was found in the lane consist of negative control—result not showed)

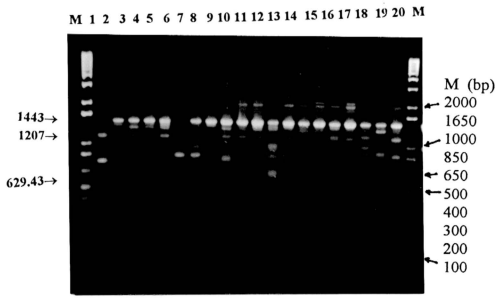


Figure 23: RAPD profile for different DNA samples isolated from *Gracilaria changii* of Morib (M) with OPA3 (Lanes 1-20). M, 1kb plus DNA ladder. (No band was found in the lane consist of negative control—result not showed)

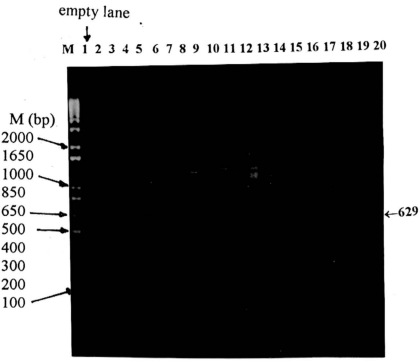


Figure 24: RAPD profile for different DNA samples isolated from *Gracilaria changii* of Ban Merbok (Bm) with OPA3 (lanes 1-20). M, 1 kb plus DNA ladder. (No band was found in the lane consist of negative control—result not showed)

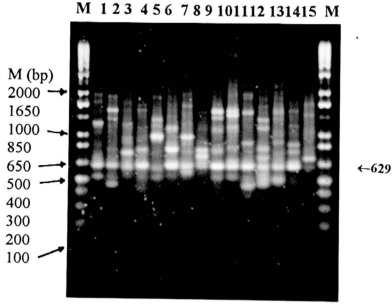


Figure 25: RAPD profile for DNA samples isolated from *Gracilaria changii* of Sungai Pulai (Sp) with OPA3 (lanes 1-15). M, 1kb plus DNA ladder. (No band was found in the lane consist of negative control—result not showed)

1 2 3 4 M 5 6 7 8

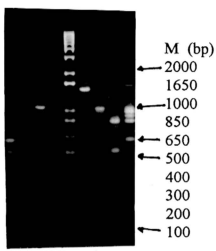


Figure 26: RAPD profile for different DNA samples isolated from *Gracilaria changii* of Phuket, P (lanes 1-4) and Takbai, T (lanes 5-8) with OPA3. M, 1 kb plus DNA ladder.

(No band was found in the lane consist of negative control—result not showed)

With OPA10, common bands of 575bp and 948bp were found in the Morib population (Figure 28) while 304bp was common in the Sungai Pulai population (Figure 30). *Gracilaria changii* collected from Carey Island and Morib which are located near to each other have a common band of 720.93bp with primer OPA10 (Figures 27 and 28).

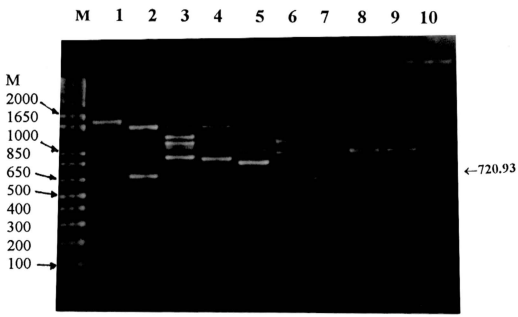


Figure 27: RAPD profile for different DNA samples isolated from *Gracilaria changii* of Carey Island (N) with OPA10 (lanes 1-10). M, 1 kb plus DNA ladder. (No band was found in the lane consist of negative control—result not showed)

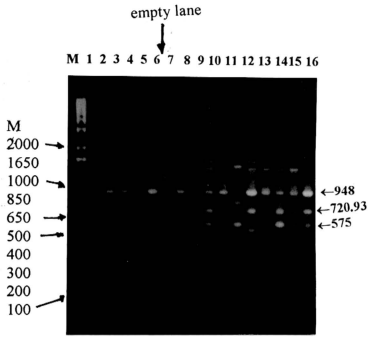


Figure 28: RAPD profile for different DNA isolated from *Gracilaria changii* of Morib (M) with OPA10 (lanes 1-15). M, 1 kb plus DNA ladder.
(No band was found in the lane consist of negative control—result not showed)

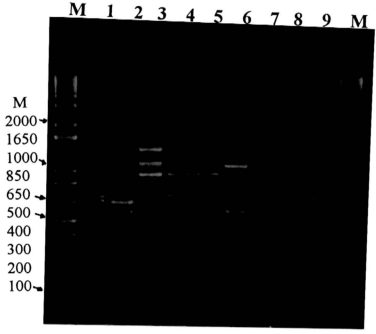


Figure 29: RAPD profile for different DNA samples isolated from *Gracilaria changii* of Ban Merbok (Bm) with OPA10 (lanes1-9). M, 1kb plus DNA ladder.

(No band was found in the lane consist of negative control—result not showed)

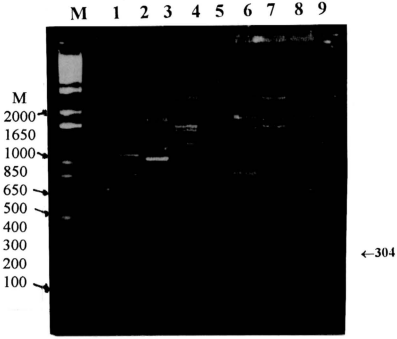


Figure 30: RAPD profile for different DNA samples isolated from *Gracilaria changii* of Sungai Pulai (Sp) with OPA10 (lanes 1-9). M, 1kb plus DNA ladder. (No band was found in the lane consist of negative control—result not showed)

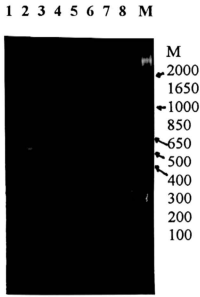


Figure 31: RAPD profile for different DNA samples isolated from *Gracilaria changii* of Phuket, P (lanes 1-4) and Takbai, T (lanes 5-8) with OPA10. M, 1 kb plus DNA ladder.

(No band was found in the lane consist of negative control—result not showed)

There was a common band of 1338bp among the *Gracilaria changii* samples collected from both Malaysia and Thailand with OPA11 (Figures 32-36). For *Gracilaria changii* collected from Thailand (Phuket and Takbai), a common band of 383.74bp was found with OPA11 (Figure 36) and 2520bp with OPA13 (Figure 41). *Gracilaria changii* collected from Carey Island and Morib which are located near to each other have three common bands of 875bp, 1055bp and 1499bp with OPA13 (Figures 37 and 38). The intensity of common bands varied with samples. Beside species-specific and population-specific common bands, there were also presence of bands specific to only one or few samples. These may provide unique fingerprints for the particular individual sample or for a group of individuals.

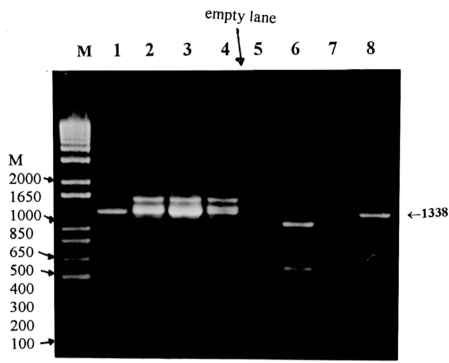


Figure 32: RAPD profile for different DNA samples isolated from *Gracilaria changii* of Carey Island (N) with OPA11 (lanes 1-8). M, 1 kb plus DNA ladder. (No band was found in the lane consist of negative control—result not showed)

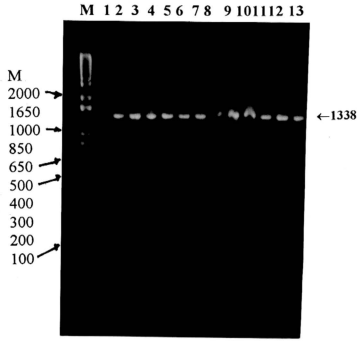


Figure 33: RAPD profile for different DNA samples isolated from *Gracilaria changii* of Morib (M) with OPA11 (lanes 1-13). M, 1 kb plus DNA ladder. (No band was found in the lane consist of negative control—result not showed)

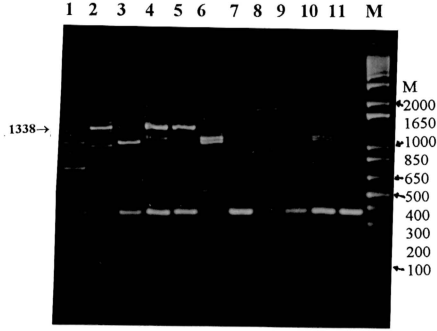


Figure 34: RAPD profile for different DNA samples isolated from *Gracilaria changii* of Ban Merbok (Bm) with OPA11 (lanes 1-11). M, 1kb plus DNA ladder.

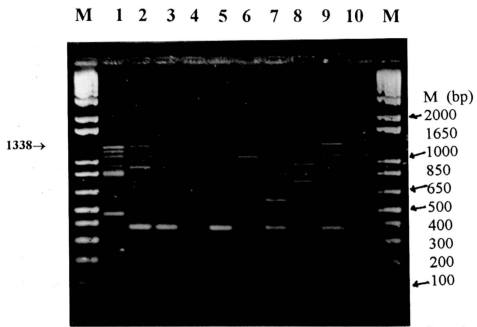


Figure 35: RAPD profile for different DNA samples isolated from *Gracilaria changii* of Sungai Pulai (Sp) with OPA11 (lanes 1-10). M, 1kb plus DNA ladder. (No band was found in the lane consist of negative control—result not showed)

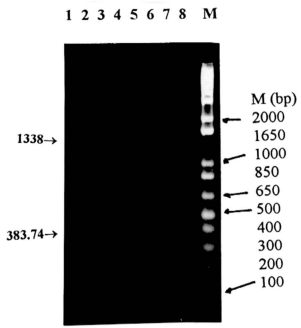


Figure 36: RAPD profile for different DNA samples isolated from *Gracilaria changii* of Phuket, P (lanes 1-4) and Takbai, T (lanes 5-8) with OPA11. M, 1kb plus DNA ladder.

(No band was found in the lane consist of negative control—result not showed)

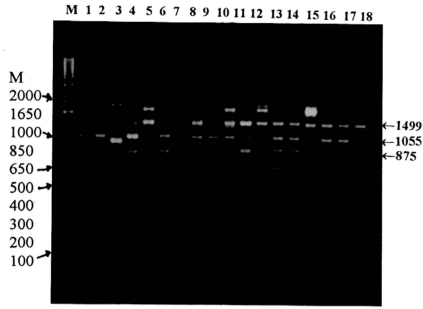


Figure 37: RAPD profile for different DNA samples isolated from *Gracilaria changii* of Carey Island (N) with OPA13 (lanes 1-18). M, 1kb plus DNA ladder. (No band was found in the lane consist of negative control—result not showed)

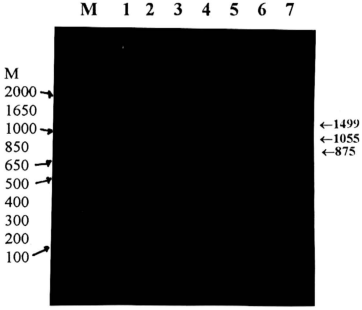


Figure 38: RAPD profile for different DNA samples isolated from *Gracilaria changii* of Morib (M) with OPA13 (lanes 1-7). M, 1kb plus DNA ladder. (No band was found in the lane consist of negative control—result not showed)

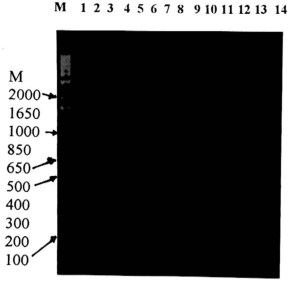


Figure 39: RAPD profile for different DNA samples isolated from *Gracilaria changii* of Ban Merbok (Bm) with OPA13 (lanes1-14). M, 1kb plus DNA ladder. (No band was found in the lane consist of negative control—result not showed)

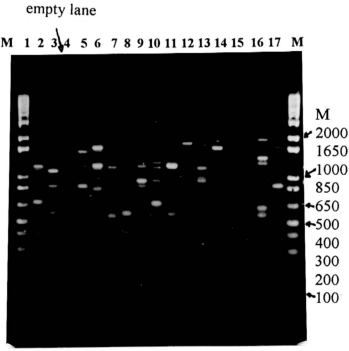


Figure 40: RAPD profile for different DNA samples isolated from *Gracilaria changii* of Sungai Pulai (Sp) with OPA13 (lanes 1-17). M, 1kb plus DNA ladder. (No band was found in the lane consist of negative control—result not showed)

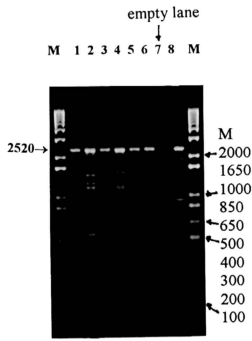


Figure 41: RAPD profile for *Gracilaria changii* of Phuket, P (lanes 1-4) and Takbai, T (lanes 5-8) with OPA13. M, 1kb plus DNA ladder.
(No band was found in the lane consist of negative control—result not showed)

4.4 DICE COEFFICIENT OF SIMILARITY (S_D) FOR INTRASPECIES RELATIONSHIP (INTRAPOPULATIONAL STUDIES)

The Dice Similarity Coefficient, S_D obtained for the *Gracilaria* samples found within the same population of each site using the four primers are shown in Appendices 1-16. Different primers gave different similarity coefficients for the same set of samples compared. There was a great variation found among individuals within a location with some samples giving S_D=0 (which means no common band found between the two samples). The results of the four primers were combined and mean values were calculated for each site (Table 7). The population of *Gracilaria changii* in Carey Island was found to have the lowest mean value indicating high level of dissimilarity within the population itself. The mean values for both populations of Morib and Carey Island were different even though these two populations are near to each other biogeographically. The Morib population has a higher similarity coefficient compared to the Carey Island population. However, the similarity coefficients among individuals found within the population were low (<50%) for each of the Malaysian populations of *Gracilaria changii* studied. As for the Thailand samples, the mean similarity coefficient obtained for both Phuket and Takbai were 0.687 and 0.765, respectively. *Gracilaria changii* samples from Thailand are therefore more similar to each other than the Malaysian samples as shown by all the four primers used.

Table 7: Dice Similarity Coefficients (S_D) obtained for each population of *Gracilaria changii* collected from different locations using primers OPA3, OPA10, OPA11 and OPA13.

COLLECTION SITES	PRIMERS	RANGE OF S_D	MEAN OF S_D
Carey Island (n=31)	OPA3	0.000-1.000	0.311
	OPA10	0.000-0.889	0.326
	OPA11	0.000-0.857	0.346
	OPA13	0.000-1.000	0.431
	COMBINED		0.353
Morib (n=30)	OPA3	0.000-0.909	0.480
	OPA10	0.000-0.941	0.587
	OPA11	0.000-1.000	0.370
	OPA13	0.000-0.909	0.500
	COMBINED		0.484
Sungai Pulai (n=34)	OPA3	0.000-0.800	0.420
	OPA10	0.000-0.900	0.387
	OPA11	0.000-0.778	0.389
	OPA13	0.000-0.769	0.359
	COMBINED		0.388
Ban Merbok (n=19)	OPA3	0.000-1.000	0.418
	OPA10	0.000-0.800	0.471
	OPA11	0.000-0.727	0.382
	OPA13	0.000-0.800	0.429
	COMBINED		0.425
Phuket, Thailand (n=4)	OPA3	0.222-0.471	0.593
	OPA10	0.222-0.500	0.620
	OPA11	0.500-0.667	0.778
	OPA13	0.471-0.667	0.755
	COMBINED		0.687
Takbai, Thailand (n=4)	OPA3	0.353-0.714	0.693
	OPA10	0.25-0.667	0.682
	OPA11	0.667-0.800	0.878
	OPA13	0.556-0.706	0.808
	COMBINED		0.765

n= number of samples

4.5 CLUSTER ANALYSIS

Different clusters using the Gelcompar software 4.1 were obtained for the four primers tested. The dendrograms generated for intrapopulational studies of Malaysian *Gracilaria changii* with the four primers are shown in Appendices 17-32.

4.5.1 Intraspecies Relationship (Interpopulational Studies)

Cluster analysis was conducted among the samples of *Gracilaria changii* collected from different populations to study the interpopulational relationship at a biogeographical level. Six samples were chosen randomly from each population in Malaysia while four samples were chosen from each of the Thailand population. They were namely N02, N06, N07, N09, N10, N12 (from Carey Island); M02, M04, M11, M12, M13, M14 (from Morib); Bm02, Bm04, Bm05, Bm06, Bm 11, Bm 13 (from Ban Merbok); Sp02, Sp03, Sp11, Sp14, Sp26, Sp27 (from Sungai Pulai); P01, P02, P03, P04 (from Phuket, Thailand) and T01, T02, T03, T04 (from Takbai, Thailand). Different cluster patterns were obtained for different primers (Figure 42-45).

4.5.1.1 OPA3

Two main groups (Figure 42) with each further divided into three clusters were observed. Group 1 consists mainly of samples collected from Carey Island, Sungai Pulai and Phuket. Cluster I in this group consists of N06, N07, Sp11, Sp26, Sp27, P01 and T04. Thailand samples (P01 and T04) was shown to have high similarity (72.5%) and Sungai Pulai samples (Sp11 and Sp27) also have high similarity (67%). In the Cluster II, N09 and N10 (Carey Island samples) have high similarity value (75%). However, both samples from Phuket (P02 and P03) were 40% similar to each other. P02 was found to have 50% similar to Carey Island samples (N09 and N10) while P03 was 59% similar to Bm02. In Cluster III, Sungai Pulai samples (Sp02, Sp03, Sp14) were in a cluster with Bm04 and P04. Sungai Pulai samples were observed to be in one group with more than 50% similarity, where, Sp03 and Sp14 were 75% similar to each other.

Group 2 consists mainly of individuals from populations of Morib, Ban Merbok and Takbai. Cluster I showed that Bm06 and M13 were 73% similar to each other while individuals from Takbai (T02 and T03) have 72 % similarity. Takbai samples were separated from the Malaysian samples in this cluster. Individuals from Morib population (M12 and M14) were grouped together with Ban Merbok samples at 45% in Cluster II while M02, M04 and M11 were grouped with Takbai sample (T1) in Cluster III.

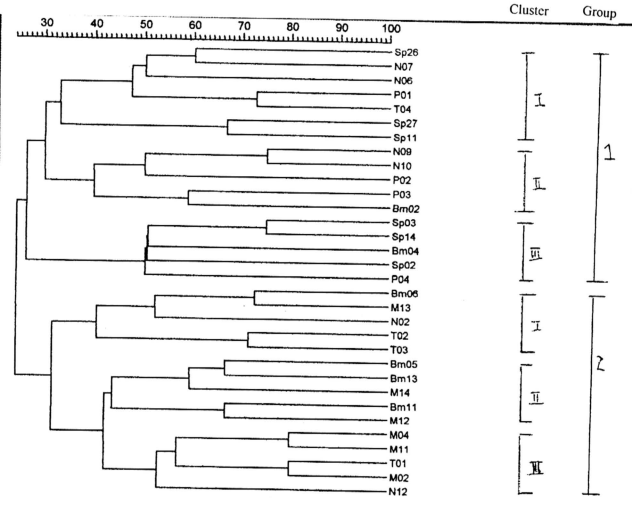


Figure 42: Dendrogram generated with the dice similarity coefficients of *Gracilaria changii* from different locations with primer OPA3 , where M, Morib, N, Carey Island, Bm, Ban Merbok, Sp, Sungai Pulai, P, Phuket and T, Takbai.

4.5.1.2 OPA10

With OPA10, N09 (Carey Island sample) was shown as an outgroup from all the other samples studied (Figure 43). The remaining samples were divided into three groups. Sungai Pulai samples was found scattered among the three groups. Group 1 was further divided into two clusters. Most of the Ban Merbok individuals (Bm02, Bm05 and Bm06) were found in Cluster I (Group 1) along with Carey Island samples (N06, N07 and N10). Morib samples were found to be in one cluster (>68% similarity to each other) in Cluster II (Group 1). Group 2 consists of individuals from different populations (M11, Sp03, Sp14, Bm04 and T04). Thailand samples were clustered together in Group 3. P01 and P03 have 52% similarity while P04 and T01 have 59% similarity. Both P01 and P03 were 32% similar to both P04 and T01. Takbai samples were found scattered among the three groups. T02 has 58% similarity to Morib samples (M02, M04, M12, M13, M14). T03 has 75% similarity to Sp02 (Sungai Pulai sample) while T04 was 55% similar to Bm04 (Ban Merbok sample).

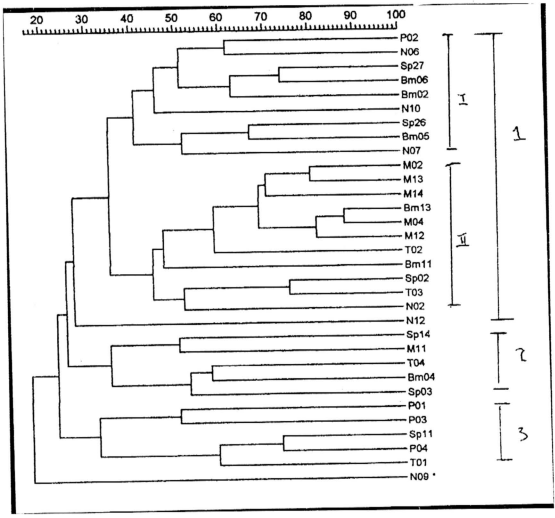


Figure 43: Dendrogram generated with the dice similarity coefficients (S_D) of *Gracilaria changii* from different locations with primer OPA10 , where M, Morib, N, Carey Island, Bm, Ban Merbok, Sp, Sungai Pulai, P, Phuket and T, Takbai.

4.5.1.3 OPA11

Two individuals from Carey Island (N6 and N10) which has 34% similarity were separated from all the other samples (Figure 44). The remaining samples were divided into three groups with Carey Island samples scattered among the three groups. Group 1 was further divided into two clusters. Cluster I consists of Sp03, Sp14, Bm06 and Bm11. Cluster II, generally was divided into three subclusters. Subcluster I consist of Morib samples (M02, M04, M12) which have 100% similarity to each other, but were 66% similar to Carey Island sample (N09). Subcluster II consists of Thailand samples (T01, T02, T03, P01, P04) which have more than 70% similarity to each other. In subcluster III, Morib samples (M11, M14) were found to be 68% similar to each other but different from the other Morib samples (M02, M04, M12) at a similarity of 52%. However, in this cluster II (Group 1), Morib and Thailand samples have a similarity value of 50%. Most of the samples from Sungai Pulai and Ban Merbok were grouped into Group 2 and 3.

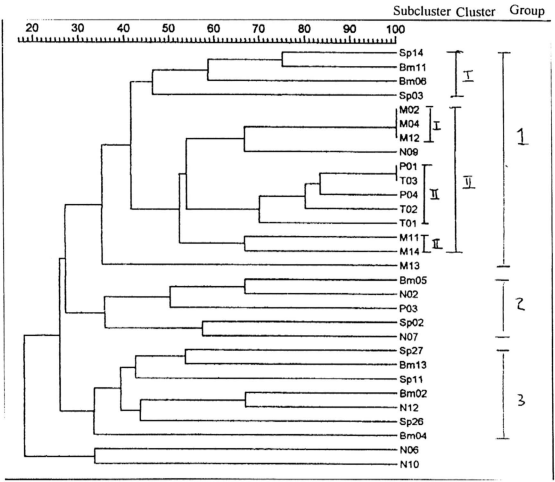


Figure 44: Dendrogram generated with the dice similarity coefficients (S_D) of *Gracilaria changii* from different locations with primer OPA11, where M, Morib, N, Carey Island, Bm, Ban Merbok, Sp, Sungai Pulai, P, Phuket and T, Takbai.

4.5.1.4 OPA13

With OPA13, Sp26 and Sp27, which were only 20% similar to each other, were separated from the other samples (Figure 45). Two main groups were observed. Group 1 mostly consists of Thailand samples (P01, P02, P03, P04, T01, T02, T04) while Group 2 consists of Malaysian samples (N02, N06, N07, N09, N10, N12, M02, M04, M12, M13). Individuals from both Ban Merbok and Sungai Pulai were found scattered in these two groups. Bm04, Bm05, Bm11, Bm13, Sp02, Sp03, Sp11 were found in Group 1 while Bm02, Bm06, Sp14, Sp26, Sp27 were in Group 2.

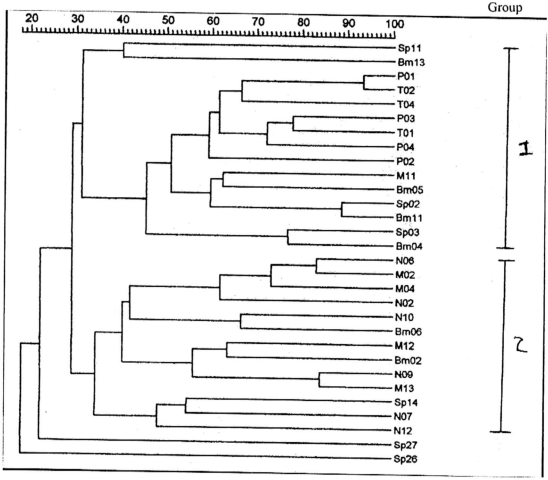


Figure 45: Dendrogram generated with the dice similarity coefficients (S_D) of *Gracilaria changii* from different locations with primer OPA13, where M, Morib, N, Carey Island, Bm, Ban Merbok, Sp, Sungai Pulai, P, Phuket and T, Takbai.

4.5.2 Interspecific Relationship

In the interspecific relationship study, cluster analysis was conducted for all the four primers used. Four samples of *Sargassum* species (Sar01, Sar02, Sar03 and Sar04) were used as an outgroup. *Gracilaria* species found in Morib were compared with each other. They consisted of *Gracilaria changii* samples (M02, M04, M11, M12, M13 and M14), *Gracilaria salicornia* samples (S03, S05 and S06) and *Gracilaria edulis* samples (E04 and E06).

4.5.2.1 OPA3

Gracilaria edulis sample (E04) was separated from the main group. Two groups were observed (Figure 46). Group 1 consists of *Gracilaria changii* (M12, M13, M14), *Gracilaria salicornia* (S03, S05, S06) and *Sargassum* (Sar02, Sar03). *Gracilaria changii* samples have low similarity (less than 55%) to each other (M12, M13, M14). *Sargassum* samples (Sar02 and Sar03) were in one group of 75% similarity. S03 and S05 have 72% similarity but were separated from S06 at 32% similarity. In Group 2, *Gracilaria changii* samples (M02, M04, M11) which has a similarity value of more than 60% were clustered with *Gracilaria edulis* (E06) at 50% similarity.

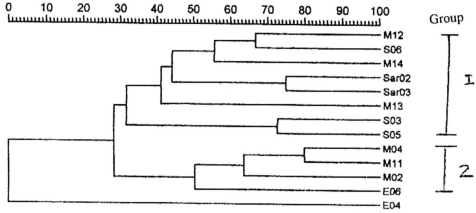


Figure 46: Dendrogram generated for *Gracilaria changii* (M), *Gracilaria salicornia* (S), *Gracilaria edulis* (E) and *Sargassum* (Sar) using OPA3.

4.5.2.2 OPA10

Gracilaria edulis sample (E06) was separated from all the other samples studied (Figure 47). The main cluster was divided into two groups. Group 1 consists of *Gracilaria salicornia* and *Sargassum* species. All the *Sargassum* species were in one cluster of more than 78% similarity while *Gracilaria salicornia* (S05 and S06) was 71.5% similar to each other. Most of the *Gracilaria changii* samples (M02, M04, M12, M13, M14) were found in Group 2 which clustered them together at a similarity of more than 68%. *Gracilaria changii* and *Gracilaria salicornia* was found together in one cluster in both Group 1 and Group 2 with a similarity value of 54% and 46%, respectively.

4.5.2.3 OPA11

Two main groups were observed with all *Sargassum* samples showed as a separate group from all the *Gracilaria* species studied (Figure 48). *Sargassum* samples (Sar01, Sar02, Sar03) were shown to have high similarity to each other (more than 70%). *Gracilaria* group was further divided into two clusters with M13 separated from the whole group. Cluster I grouped some of the *Gracilaria changii* samples (M11 and M14) together with *Gracilaria salicornia* (S03, S05, S06). Other *Gracilaria changii* samples (M02, M04, M12) were grouped together with *Gracilaria edulis* (E06) in Cluster II.

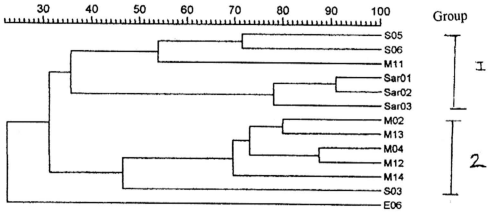


Figure 47: Dendrogram generated for *Gracilaria changii* (M), *Gracilaria salicornia* (S), *Gracilaria edulis* (E) and *Sargassum* (Sar) using OPA10.

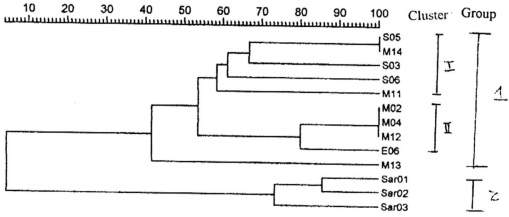


Figure 48: Dendrogram generated for *Gracilaria changii* (M), *Gracilaria salicornia* (S), *Gracilaria edulis* (E) and *Sargassum* (Sar) using OPA11.

4.5.2.4 OPA13

Two main groups, which were further divided into two clusters were observed (Figure 49). In Cluster I (Group 1), *Gracilaria salicornia* (S03, S05, S06) were clustered together with *Gracilaria edulis* (E06). Cluster II (Group 1) consists of all the *Gracilaria changii* samples (M11, M12, M13) at a similarity of more than 61%. Both Cluster I and Cluster II in Group 1 are 41% similar to each other. In Group 2, Cluster I consists of *Gracilaria changii* samples (M02 and M04). All *Sargassum* species (Sar01, Sar02, Sar03 and Sar04) were clustered into one group of more than 72% similarity in Cluster II (Group 2). Both Cluster I and Cluster II in Group 2 are 57% similar to each other.

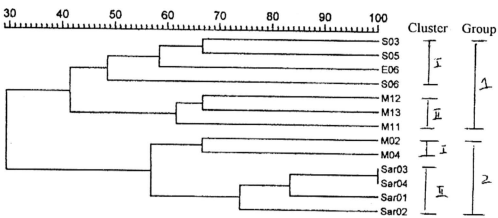


Figure 49: Dendrogram generated for *Gracilaria changii* (M), *Gracilaria salicornia* (S), *Gracilaria edulis* (E) and *Sargassum* (Sar) using OPA13.

4.6 SUMMARY OF RESULTS

1. Morphological differences between *Gracilaria changii* from different locations were observed viz difference in colour and thickness of thallus.
2. DNA extraction and purification-Protocol 2 was selected because the DNA isolated has higher purity and yield compared to Protocol 1.
3. Optimisation conditions for PCR – Annealing temperature at 38°C was selected as polymorphic band patterns were obtained with much sharper bands (Optimised condition).
4. RAPD band patterns are reproducible under the same conditions with high quality of DNA template.
5. Of 20 primers screened, four primers: OPA3 (AGTCAGCCAC), OPA10 (GTGATCGCAG), OPA11 (CAATCGCCGT) and OPA13 (CAGCACCCAC) were selected for generating RAPD profile for comparison.
6. OPA3 gave a common band of 629.43bp among the *Gracilaria changii* collected from Malaysia.
7. OPA3 gave common bands of 1244bp and 1660bp for Carey Island samples.
8. OPA3 gave common bands of 1207bp and 1443bp for Morib samples.
9. OPA10 gave common bands of 575bp and 948bp for Morib samples.
10. OPA10 gave a common of 304bp for Sungai Pulai samples.

11. OPA10 gave a common band of 720.93bp for samples from both Morib population and Carey Island population.
12. OPA11 gave a common band of 383.74bp for samples collcted from Thailand (Phuket and Takbai).
13. OPA13 gave a common band of 2520bp for samples collected from Thailand (Phuket and Takbai).
14. OPA13 gave common bands of 875bp, 1055bp and 1499bp for samples collected from both Morib population and Carey Island population.
15. Comparison of the dice similarity coefficients (S_D) using the four primers suggests that *Gracilaria changii* samples from Thailand have higher similarity than the Malaysian samples. There is significant intrapopulational variation among the individuals found in one population. The Carey Island samples in particular have a very dissimilar population.