CHAPTER 5: DISCUSSION

5.1 DEOXYRIBONUCLEIC ACID (DNA) EXTRACTION AND ISOLATION

Gracilaria samples, which were collected from the mangroves or fishpond, were contaminated with mud, salt and epiphytes. Hence, *Gracilaria* must be cleaned with great care. Unclean and heavily epiphytised specimens were usually discarded. Although with careful selection and cleaning of the *Gracilaria* samples, there may be presence of endoparasite which cannot be detected by naked eyes (George, 1998). *Gracilaria* samples studied were checked randomly under microscopic inspection to minimise this problem. Cleaned tissues were then dried and ground using liquid nitrogen. It was found that grinding dry sample to powder form was easier than grinding the wet sample. Thorough breakage of the tough cell walls occurred with use of dry samples. Saunders (1993) emphasised the importance of immediate drying of the fresh sample and storage in a dry environment. Doyle and Dickson (1987) reported that dried material of preserved higher plant tissue gave the best results for DNA isolation.

Sinnappah (1994) tried six different methods of DNA extraction to isolate DNA from *Gracilaria* species. She reported that the CTAB and phenol chloroform combination proved to be the most effective method. The range of

$OD_{260/280}$ she obtained for the DNA samples was 1.51-3.56. The range of DNA concentration isolated through this protocol was 1.2-12.1μg/g. Protocol 1 (this present study) used the CTAB and Phenol extraction protocol adapted from Sinnappah (1994). The range of $OD_{260/280}$ obtained for the *Gracilaria* DNA samples obtained in this study was 1.00-1.57 while the DNA yield was 3.33-5.67μg/g.

A modification of this protocol was carried out. Protocol 2 (this present study) applied the same CTAB and phenol combination as in Protocol 1, with additional steps of DNA isolation from the residue and a reextraction step (Figure 5). In Protocol 2, further extraction of DNA from this residue (which was discarded in Protocol 1) was carried out. DNA isolated was further purified by reextraction using 10% CTAB, which remove the unwanted polysaccharides. Comparison of the quality and yield of the DNAs isolated from both Protocol 1 and 2 are shown in Table 5. Protocol 2 was found to give higher purity with better yield of DNA. It is suspected that most of the DNA was found in the residue after the protease treatment step. Table 6 shows the quality of DNA isolated from *Gracilaria changii* collected from different locations using Protocol 2. One of the main problems faced in isolating DNA from *Gracilaria* is the presence of polysaccharide contamination as *Gracilaria* is a high agar producer. This may be the reason for the low value of $OD_{260/280}$ and the inconsistency of this value among the samples. This was also observed with *Sargassum* (Ho, 1994; Ng, 1998). Another cause of inconsistency of the purity and yield of DNA is the

different contents of the polysaccharide produced by each sample. If the sample collected has high polysaccharide content, it will produce low purity and low yield of DNA. One of the suggestions to overcome this problem is to keep the sample in the dark overnight before harvest for DNA extraction to reduce the starch content in the tissue. Other factors affecting the quality and yield of DNA obtained are the type of species collected and also the developmental stage of each individual. Mayes *et al.* (1992) reported that the selection of alternate life-history stages is important as DNA yields from microscopic gametophytes and meiospores are greater compared to yields from macroscopic sporophytes. Less polysaccharide concentration in gametophytes and meiospores minimises the problems of organelle loss and nuclease degradation of DNA during extraction.

5.2 RANDOM AMPLIFIED POLYMORPHISM DNA (RAPD)-

5.2.1 Optimisation of PCR Reaction

Among 20 primers of Operon kit A (Operon Technologies, California, USA) screened, four primers (OPA3, OPA10, OPA11 and OPA13) were selected and used in the molecular taxonomic studies of *Gracilaria* species. The effect of annealing temperature to the RAPD reaction was assessed at 33°C, 36°C, 38°C and 40°C (Figures 17-20). It was found that the number of bands varied with the annealing temperature. Higher temperature was observed to give more bands compared to lower annealing temperature. The reduction in the number of bands

at lower annealing temperature may be caused by the occurrence of some mismatched primer-template combinations under low-stringency annealing conditions which competed with the perfectly matched sites (Williams *et al.*, 1990, Ruano *et al.*, 1991). This is further supported by the presence of bands with varying intensity in the profile. Ng (1998) also reported in her studies that different annealing temperature is applied to different primer for optimised RAPD amplification. However, in this study the optimised annealing temperature for the four primers were almost the same. The annealing temperature at 38°C was then selected for all the subsequent RAPD reactions.

Yu and Pauls (1992) showed that optimisation can also be applied to the duration time for each step and the number of cycle, which will reduce the length of time needed for the PCR program. Wilkie *et al.* (1993) observed that the stringency of PCR amplification is influenced by the magnesium ion concentration which should be as low as possible, and the annealing temperature which should be as high as possible. Many other conditions such as source of *Taq* polymerase brand, type of thermal cycler and primers are important factors in obtaining good DNA amplification. Unfortunately, optimal species-primer combinations have to be found by trial and error. The GC content of the desired primer and its optimal annealing temperature are not correlated. Therefore, optimising PCR-technology is laborious as each parameter involved affect the reaction (Wolff *et al,* 1993).

Finally, different staining methods can be used to detect the amplified bands. The resolution and sensitivity achieved by agarose gel electrophoresis and ethidium bromide staining can serve to reduce the apparent complexity of the band profile while the utilisation of the high-sensitivity silver-stain detection of amplified bands result in more complex band profiles (Caetano-Anolles *et al.*, 1991), which may be more discriminative.

5.2.2 Reproducibility

Several sets of RAPD reactions were run on identical DNA samples from *Gracilaria changii* with the same primer OPA10. The same combination of primer-DNA template gave similar bands patterns (Figure 21). Most of the major bands were reproducible for most of the reactions while minor bands were variable. Variation in the intensity of individual bands was also observed with different amplifications of the same template-primer set. Gehrig *et al.* (1997) also reported the same observation. The different efficiency of amplification among the heating block wells in the thermal cycler used may cause the difference (Patwary *et al.* 1993). Different efficiency at different wells will affect the temperature-sensitive RAPD reaction and produce variation in the band patterns even though the same DNA template-primer sets were used. According to Linz (1990), the variation in efficiency of amplification among the heating block wells of the thermal cycler may also cause variable results in RAPD as it affects the annealing temperature. Hence, the use of different thermal cyclers may affect the

result depending on its temperature sensitivity (Klein-Lankhorst *et al.*, 1991). Chen *et al.* (1997) also found problems when different batches of synthesized oligo-primers and different thermal cyclers were used to check the reproducibility of RAPD.

However, in this study, RAPD reactions were observed to be reproducible. As the minor bands (bands that were unclear and faint) were not considered in the calculation of similarities coefficient, it will not give much influence to the objective of this study. Ho *et al.* (1995a) also reported on the good reproducible RAPD band patterns for the discrimination of individual and relationship studies between species. As RAPD reaction is a very sensitive technique, pure DNA is essential for reproducibility of RAPD analysis (Mizukami *et al.*, 1998).

Result from the present study confirms that RAPD gives good reproducible band patterns, which can be used in discriminating individuals, developing individual fingerprints and determining relationships between species if all the parameters are kept constant and pure DNA is used as template.

5.3 INTRASPECIFIC RELATIONSHIP (BIOGEOGRAPHICAL ASSESSMENT)

RAPD profiles were generated for DNA isolated from *Gracilaria changii* collected from different locations using primers OPA3, OPA10, OPA11 and OPA13 (Figures 22-41). Some common bands were observed along with some specific bands. The intensity of common bands varied with samples. Wilkie *et al.* (1993) attributed this to the copy-number polymorphisms or amplification efficiency.

Baril *et al.* (1997) reported that common markers could be used to predict the value of crosses in improving breeding program. Useful phylogenetic information can be gained from an analysis of common bands, but it is important to ensure that those bands, which co-migrated in different species, represented homologous sequences, and were genuinely common and not simply fortuitous bands of similar size. Southern analysis of RAPD gels using single isolated bands as probes can provide information on the degree of homology between common bands. In 1997, Dubouzet *et al.* purified the randomly amplified DNA sequences by gel excision and used them as probe in dot blot analysis to produce genetic markers for phylogenetic studies of various species of *Allium.*

As for unique bands found for each individual or group of individuals, they can contribute to the detection of genomic regions that cause heterosis of the

traits of interest. Curtsinger *et al.* (1998) successfully identified genetic markers for life span using RAPD while Emebiri *et al.* (1997) detected the quantitative trait loci (QTLs) that linked to needle-to stem unit rate (NESTUR), a stem growth index of conifer seedlings.

Hence, RAPD can be used for a wide variety of studies even to the possibility of developing individual fingerprints for each individual sample. Hardys *et al.* (1992) reported that discrimination between individuals, strains, or species could be done through the DNA fragment patterns generated. However, Schierwater and Ender (1993) stated that RAPD markers identified in different laboratories are not interchangeable unless all the conditions of the RAPD reaction are the same. King *et al.* (1993) reported that its sensitivity to reaction conditions which are not always easy to control cause the major drawback of its application.

5.3.1 Intrapopulational studies

Comparing the mean values of the similarity coefficients among all the locations, *Gracilaria* samples located in Carey Island was found to have the lowest similarity value (Table 27), which indicates that there is a wide variation among the individuals within this population of *Gracilaria changii* growing on Carey Island. Samples collected from Carey Island were mostly healthy vegetative plants. Most probably under the favourable environment found in

Carey Island, *G. changii* propagates vegetatively for it is faster than sexual reproduction. Meneses *et al.* (1998a) reported that fragments obtained from the same thallus demonstrated changes after a certain period of growth. Meneses *et al.* (1998b) observed genetic variability, especially at the active growth apical, among fragments of the same thallus. Rapid replication may cause some genetic changes within the same plant. Chen *et al.* (1997) reported that there are some differences between leaf and root DNAs within a line shown by the comparison of RAPD patterns. They further confirmed that the amplified bands were homologous with the total genomic DNA using southern hybridisation.

Although Morib and Carey Island are located near to each other, the values of intrapopulational variation between these two sites were not the same. Morib has higher similarity value compared to Carey Island (Table 27). In Morib, besides *G.changii*, both *G. edulis* and *G. salicornia* grow in this mangrove. Most of the *G. changii* samples collected from this site were fertile plants with carpospores growing on them. Coyer *et al.* (1997) reported that genetic relatedness decreased with increasing geographical distance depending on whether asexual reproduction or sexual reproduction was common at the particular population. Diploids have a higher survival rate in a competitive environment and intraclonal variation may be less obvious although it does take place. The sequence differences among the individuals in a reproductive population may be less compared to a non-reproductive population. However, when using RAPD technique, the ploidy of the samples has to be considered.

Diploid individuals will have more primer binding sites with its double sized-genome compared to haploid individuals. Hence, the competition for primer-template binding sites is higher in diploids which then reduces the number of bands generated (Meneses, 1996). Another cause of intrapopulation variation may be the different growth phases exhibited by *Gracilaria*. Apart from the normal life history, Kain and Destombe (1995) found that some plants of *Gracilaria* bear both gametangia and tetrasporangia where gametophytic thalli develop as epiphytes on tetrasporophytes. They also observed some haploid male plants turned into bisexual plants.

In addition, mutation was also observed with *Gracilaria* species. Van der Meer and Zhang (1988) detected unstable mutations in three species of *Gracilaria*, which may be caused by transposons. They reported that in all the three species of *Gracilaria*, the green mutants frequently reverted to wild type during somatic growth of the haploid plants, giving numerous flecks and larger patches of red tissue on the green plants. The wild-type revertant tissue appeared completely stable and could not be distinguished from those normal plants. The unstable green phenotype in each of the three species was found to be recessive where the majority of the resultant F_1 tetrasporophytes from crosses between wild-type plants and unstable green mutants, had wild type colour. Wattier *et al.* (1997) also observed a high mutation rate among *G. gracilis*. They found that high level of genetic variability within *Gracilaria gracilis* and single locus microsatellites revealed a high level of within-population polymorphism in two of

the loci identified. They reported that the extraordinarily large number of alleles observed at locus GvICT indicated exceptionally high mutation rate. Globally, it is also been observed that a higher proportion of step-wise mutation happened at the intraspecific level compared to the interspecific level. Mutation, which is very common among *Gracilaria* species, may be the reason for the intrapopulational variation at the different locations tested especially in Sungai Pulai. Sungai Pulai is located at the southern part of Peninsular Malaysia and it is being developed into a jetty. Under the harsh environment, some mutations may occur for survival at the threat of extinction. Although any two cells randomly chosen from an individual are likely to be identical at a locus, there are so many cells within an individual and so many genes than can mutate, hence implying the existence of significant genetic variation within an individual. Selection within the individual causes evolutionary change to a population and acts as a selective sieve, promoting beneficial mutations favoured by environmental demands while eliminating deleterious ones. Otto and Hastings (1998) studied the germline selection which occurs when cells that differ genetically (because of mutation, crossing over, or gene conversion occurred during mitosis) also differ in their propensity to proliferate or survive during development. They reported that when a genetic mosaic is created by mutation, the frequency of the mutation transmitted to offspring depends on whether the mutation increases or decreases cell proliferation. Mutation that increases the cell proliferation will be more likely to be transmitted. The greater the number of cell divisions, the more selection will occur within an individual generation. Tomiuk *et al.* (1998) also gave a report on

the estimation of population differentiation through mutation and drift. Moreover, Sungai Pulai is situated near Singapore, there may be a mix population between Malaysian and Singaporean *G. changii*.

Apart from the wild samples, cultivated *G. changii* was collected from Ban Merbok. Morphologically, Ban Merbok samples were thinner but longer and grow in a big bunch. The colour of the plants is brownish to yellowish because the plants grow under the water surface of the fishpond and less exposed to the sun. Individuals in the Ban Merbok population were found to have low similarity values too. Due to different sources of seedlings for the cultivation purpose from time to time, Ban Merbok may have a mixture of populations among the samples. Dehghan-Shoar *et al.* (1997) also reported that all individual seedlings within cultivars were found to be genetically distinct using RAPD. They observed that very few bands were common to all the profiles for the 40 seedlings of Lucerne cultivar from the two Iranian cultivars either Azari or Bami. They found differences between cultivars. Since all the samples collected from Ban Merbok were vegetative plants, it was compared with the wild vegetative plants of Carey Island. The intrapopulational variation in Ban Merbok was found to be lower than that at Carey Island. *G. changii* in Ban Merbok grow in a fish pond where conditions are more stable compared to the wild environment of Carey Island. Chan and Sun (1997) stated that high levels of genetic variability are expected in the wild species, as it will also facilitate the natural selection for survival. Hence, wild species has higher level of polymorphism compared to cultivated species.

As a whole, the result of the intrapopulation studies seemed contradicting to the hypothesis that the material collected from the same geographic region has higher levels of similarities as selections take place in the same natural populations (Paul *et al.*, 1997). However, Wattier *et al.* (1997) detected high level of within- population polymorphism in *G.gracilis* using microsatellite loci and Klekowski (1998) reported that mutation rates per cell division was not constant but vary within species. Santelices and Varela (1993) reported that there are morphological variations among clones of *Gracilaria chilensis* grown under similar environment. Santelices *et al.* (1995) reported that there is significant intraclonal variation in *Gracilaria* and several red algal species where plants derived from the same clone were found to have different growth responses even under similar conditions.

Generally, intraclonal variation is the main cause for the dissimilarity among the samples of each population. Intraclonal variation was found in all stages of the life history of *Gracilaria* and there are two factors that cause genetically intraclonal variation. Firstly, genetic instability such as somatic mutation or somatic recombinations is common in spores of *Gracilaria* (Van der Meer and Todd, 1977). Other kind of genetic changes are the presence of mobile genetic elements (transposons) and mitosis errors or intragenomic recombinations (Santelices *et al.*, 1995). Secondly, sporeling coalescense (Munoz and Santelices, 1994). Spores of species of *Gracilaria* when grow together in a form of a completely coalesced mass has the ability to develop into a single plant (Maggs

and Cheney, 1990). The occurence of unitary thalli that in fact correspond to genetically different coalesced individuals was further confirmed by Santelices *et al.* (1996) with their studies on unisporic and polysporic thalli using transmission electron microscopy (TEM) and RAPD data. Under TEM, these polysporic plantlets which are formed by groups of cell clusters appeared as discrete units surrounded by a common external cell wall. These polysporic plantlets were formed by groups of spores and their derivatives. They observed that even though adjacent cells in two different groups may fuse, these groups maintained an independent capacity to grow and form uprights. The indication of the chimeric nature of the coalesced individuals was the construction and growth of bicolour individuals (bicolour holdfasts had green and red cells). They further studied the existence of these mixed tissues using RAPD analysis and found that the banding pattern produced by branchlets of an unisporic thallus was consistently monomorphic, whereas the patterns produced by the polysporic thallus were polymorphic. Growth rates of these polysporic thalli were observed to have larger data dispersal and variation coefficients than oligosporic or monosporic thalli.

5.3.2 Interpopulational Studies

RAPD was used to assess the genetic variation among the *Gracilaria changii* collected from different locations. RAPD patterns generated seemed unable to form a relationship at geographic scales with most of the primers tested (OPA3, OPA10, OPA11). Samples of the same population were scattered around and not in one cluster (Figures 42, 43 and 44). In this study, populations located near to each other did not have high similarities as reported by Gonzalez *et al.* (1996). They analysed organellar DNA (plastid DNA) restriction fragment length polymorphism (RFLP) of the morphotypes of *Gracilaria* from two different locations (Maullin and Lenga) and reported that the DNA banding patterns were identical irrespective of morphotypes or locations. However, in their attempt to unravel the nature of the morphological differences found among these morphotypes using RAPD analysis of nuclear DNA, no polymorphism has yet been obtained. Intasuwan *et al.* (1993) also reported that the shortest geographic distances between populations of *G. chilensis* Bird, McLanchlan & Oliviera gave the smallest genetic distance in allozyme variation, but, they found that the maximum geographic distance between New Zealand populations did not occurr with the largest genetic distance. They reported that the UPGMA cluster analysis separated the populations into two groups, a northern group and a group found throughout the country. They also reported that the genetic distances between populations ranged from 0.00 to 0.43. However, Van Oppen *et al.* (1995) reported on their studies of genetic variation within and among populations of

Phycodrys rubens, there was high levels of variation easily identified by RAPD data but not detected by allozyme technique or spacer sequences. As RAPD applied to total DNA it has high probability of detecting genetic variability even to the level of intrapopulational variability. Sane *et al.* (1997) reported that RAPD technique was able to detect the variability in the mitochondrial genomes, which lead to a better characterisation of the organelle genome diversity. The detection of variation in cytoplasmic genomes, especially the mitochondrial genomes, is important in cytoplasmic male sterility (CMS) lines. Hence, the inability of RAPD data forming a relationship at geographic scales was not mainly caused by artifacts, but the high amounts of drift within small populations and may be disruptive selection. In their studies on the allozyme variation in *Parnassius mnemosyne* populations, Meglecz *et al.* (1997) reported that no correlation between population variation and geographic distance as the level of genetic differentiation was similar between adjacent populations and between populations originating from different geographical regions. They found that the average genetic distances within regions were similar or even higher than those averaged between regions. This indicates that the differentiation between regions was not stronger that between populations within a region. They also observed that populations from the same region were not clustered into a single branch. They suspected genetic drift was the main evolutionary force in the populations.

Primer OPA 13 managed to divide the samples tested into two groupings: Malaysia and Thailand (Figure 45). Most of the Malaysian samples were

clustered together except for a few which were found scattered. However, this primer was unable to further cluster the Malaysian samples according to their populations. In 1996, Van Oppen *et al.* reported that "noise" (result of technical artifacts) affected the RAPD data of small biogeographical scales but not the large biogeographical scales where the phylogenetic signal is strong. They also stated that intrapopulation variation may become as large as interpopulation variation and no geographical boundary can be assumed. They reported that the presence of tetrasporophytes and gametophytes in a sample is not important in large-scale phylogeographic studies but does affect within-clade variation at smaller scales. Southern analysis using individual RAPD bands as probes revealed that 16% of visually non-detectable bands are actually present. However, they found that the overall level of error scored remained constant at 5 and 10%, which may not be a problem at large biogeographic scales where the phylogenetic signal is strong. They also observed that at large-scale biogeographic comparisons, nested-band artifacts (an over estimation of similarities because bands will be scored more than one time) are not likely to affect basic group discrimination as the overall number of synapomorphies contributing to the backbone topology will outweigh the few extra counted synapomorphies.

Generally, in this interpopulation study, the S_D value ranged from 0-1 (Table 7, page 88). Some of the samples were found to have S_D=0, which means there are no common bands between the two samples being compared while S_D=1 meaning that the obtained patterns are identical. In their studies of RAPD

analysis on rice, Sane *et al.* (1997) reported that some of the primers (OPA11 and OPB7) resulted in a highly polymorphic profile with no similarity amongst the individual lines, but, the profile generated from OPA12 was mostly monomorphic. Gehrig *et al.* (1997) reported that RAPD fingerprinting gave interesting information in the study of individual *Kalanchoe* species. *Kalanchoe beharensis*, which exists in two forms, having leaves densely covered with brown hairs or has naked, waxy leaves, was observed to be the same species demonstrating different phenotypes. They reported that with some primers applied, they obtained for both forms of *Kalanchoe beharensis*, identical patterns of amplification products, but with others primers, the amplification patterns were however, significantly different. As observed by Bird *et al.* (1992), the sequence variability within morphological similar *Gracilariopsis* (individual level) from the Pacific and European populations showed values found between some genera. Dehghan-Shoar *et al.* (1997) reported that although discrimination of 99% probability level among the majority of the ten cultivars (40 seedlings each) with single primer, some pairs could not be discriminated using the same primer. In their assessment of 22 more primers, they found that primers vary markedly in their power to generate fragments, which are polymorphic between Lucerne cultivars. Intraspecific variation existence was also reported in cotton (Iqbal *et al.*, 1997) and *Amaranthus* (Chan and Sun, 1997).

However, RAPD technique can still be applied to biogeographic studies depending on the random primers used. In her studies on the populations of *G.*

chilensis, Meneses *et al.* (1996) reported that RAPD is able to cluster the samples from two different populations despite their different morphologies. If a more conserved region was being amplified, a higher degree of similarity will be obtained, but, if a more variable region was being amplified, it will show great variation among the samples. The primers used in these studies demonstrated high degree of variability among the samples and may be useful in differentiating each individual. However, the homologous nature of the bands generated has to be confirmed using techniques such as southern hybridisation.

Examination of a wider range of samples within each population is desired to generate a more accurate dendrogram. However, with a great amount of samples, a complicated dendrogram will be obtained due to artifact and genetic variation of each individual. It is suggested to use bulk genomic DNA as a sample of the population DNA for RAPD reaction. Alberto *et al.* (1997) concluded that using this method, large sample sizes can be screened at a relatively low cost and less time consuming and it is applicable to RAPD in assessing the genetic similarity among *Gelidium sesquipedale* populations. Emebiri *et al.* (1997) used bulked segregant analysis in conjunction with RAPD markers for tagging and identifying specific genes or quantitative trait loci (QTLs).

5.4 INTERSPECIFIC RELATIONSHIP

RAPD was also used to study the interspecific relationships among *Gracilaria* species (*G. changii, G.salicornia* and *G. edulis*) and *Sargassum* was used as an outgroup. OPA 3 did not amplify the conserved region in the genus *Gracilaria* as all the *Gracilaria* species were not clustered together and *Sargassum* was not shown as an outgroup (Figure 46). However, it showed higher similarities values of the samples tested compared to other primers.

With OPA 10, *G. salicornia* was shown to be more similar to *G. changii* compared to *G. edulis* (Figure 47). Millow and Salleh (1994) carried out taxonomic studies on the three species of *Gracilaria* from Morib and concluded that they have distinct morphological and anatomical characteristics. *G. changii* and *G. salicornia* were reported to possess characteristics that matches those of *Gracilaria changiii* (Xia et Abbott) Abbott, Zhang et Xia while *G. edulis* has morphological characteristics which match with *G. edulis* (Gmelin) Silva. Ho *et al.* (1994) also reported that *G. edulis* and *G. changii* were dissimilar to each other, but they were found closely related to other groups of algae tested.

OPA 11 was found useful in differentiating the samples tested (Figure 48). *Sargassum* was shown as an outgroup from the *Gracilaria* species *Gracilaria changii* was further divided into two groups. One group showed higher similarities to *G. salicornia* while the others with *G. edulis*. There may have been

some hybridisation occuring between these species as they are growing in the same locations. RAPD markers were successfully applied in the detection of hybrids. In 1997, Bommineni *et al.*, identified RAPD markers specific to some perennial grasses and durum wheat cultivars and applied both RAPD and RFLP markers for the detection of *Thinopyrum junceiforme* specific DNA fragments in its hybrids with durum wheat. They confirmed the presence of the 'alien' fragments by southern hybridisation. In 1988, Pacak *et al.* used RAPD technique in the genomic relationship studies of the genus *Pellia*. The RAPD analysis supports the distinction of the sibling species of *Pellia epiphylla*-species N and *Pellia epiphylla*-species S, which have an allopatric distribution in Poland (N-North, S-South Poland). Their data also showed a hybrid origin (alloploid) of the polyploid *Pellia borealis* from *Pellia epiphylla*-N x *Pellia epiphylla*-S. They found that 30 DNA fragments were shared between *Pellia epiphylla*-N and *Pellia borealis* and 34 DNA fragments between *Pellia epiphylla*-S and *Pellia borealis*. There was no species-specific DNA fragment observed for *Pellia borealis*. The distance between *Pellia epiphylla*-N and *Pellia epiphylla*-S was 0.33 while *Pellia borealis* was very close to all *Pellia epiphylla* populations (0.09-0.03).

In the present study, the dendrogram generated based on RAPD data using OPA13 gave good separation of the *Gracilaria* species from *Sargassum* (Figure 49). However, there were some samples found scatter. The presence of artifacts or contamination may cause this problem. This primer also showed that there was a relationship between *G.edulis* and *G. salicornia*.

These results show that RAPD can be applied for interspecific studies and in the detection of hybrids. RAPD is good as a molecular tool for interspecies relationship studies where the phylogenetic signal is strong and is able to overcome the effect of artifact or error. Baril *et al.* (1997) showed that using RAPD in calculating the genetic distance is useful in differentiating the two species of *Eucalyptus*. They reported that the contingency table of the number of markers contains common markers with 226 RAPD variables (or 54.5% of the total number of markers) and the specific markers with 189 RAPD variables (or 45.5% of the marker sample). Ninety-nine of these specific markers are more frequent in the *E.urophylla* population while 90 are in the *E. grandis* population. They observed that the amount of variability corresponding to the specific genetic distance is greater within species than between species and the mean distances within *E. urophylla* are systematically greater than the mean distances found within *E. grandis*. However, the mean distances between species are clearly greater than the mean distances within species. Moreover, RAPD markers have been successfully used for the estimation of genetic similarities of cotton varieties (Iqbal *et al.*, 1997). Gehrig *et al.* (1997) also reported that RAPD-fingerprinting provides a quick and sensitive tool useful for plant phylogenetic relationships studies at the intrageneric level. In their studies on the genus *Kalanchoe*, they found that the species form three main clusters correlated with the three intrageneric groups of species distinguishable by their mode of Crassulacean acid metabolism (CAM) performance and by classical taxonomic criteria.