## 6.0 CONCLUSION

Molecular data produced in this thesis clearly indicates that *S. binderi* and *S. oligocystum* are not recommended as synonyms as reported by Womersley and Bailey (1970) but are separate species as proposed by Ajisaka (1998). To differentiate between the two *Sargassum* species which are morphologically alike, the vesicles are the important feature to consider as the vesicles of *S. binderi* are winged but *S. oligocystum* are not. Another feature to determine the difference between the two *Sargassum* species are the receptacles. *S. binderi* has flattened, twisted, receptacles with sharply dentate margins but the receptacles of *S. oligocystum* do not.

The use of a modified simple technique for DNA isolation from fresh plant tissue (Doyle and Doyle, 1990) successfully yielded high quality, intact genomic DNA. This method is fast (fewer steps involved), cheap, easy to handle and less toxic than the commonly used methods that requires phenol / chloroform extraction steps. Protocol 2 was chosen to extract all the 60 samples as the DNA obtained was clear, non-pigmented, intact and the quality of the DNA was much more improved when re-extraction was carried out. From the *Sargassum* samples, only young shoots and leaves were used for the DNA extraction as these were the parts that were easily cleaned from contaminants and also mitosis is involved in the young shoots. The receptacles were not used as they have uneven and twisted surface which were very

difficult to clean from the contaminants of diatoms and epiphytes although meiosis was actively involved in the receptacles whereby DNA was doubled during the meiosis.

This study demonstrates that the RAPD methodology is very powerful for the discrimination of the two *Sargassum* species, that is, *S. binderi* and *S. oligocystum* at the species level. The results indicate that the RAPD method using OPA13, OPK7, OPK9, OPN6 and OPN16 yields amplification products which are species-specific for the samples investigated through the phylogenetic analysis. OPN16 is not a very good primer to separate out samples of *S. binderi* or *S. oligocystum* intraspecifically. Therefore, it is not advisable to use it as a probe for DNA fingerprinting. OPK9 is the best primer among the five as this primer is not only can separate out the samples species-specificly but also the individuals of the two *Sargassum* species. It should be possible to recover these PCR bands and employ them as molecular probes or develop primers from them to screen the respective *Sargassum* species specificly as mentioned above. For OPA13, OPK7 and OPN6, these primers can use to screen a large amount of the respective *Sargassum* samples rapidly interspecifically but not individually. Despite these promising results, caution has been recommended in the use of RAPDs for macroalgae since several technical limitations could contribute to produce differences in banding patterns due to other factors rather than sequence differences.

Although the results obtained demonstrates that the RAPD methodology is robust to characterise *S. binderi* and *S. oligocystum*, the more general utility of these DNA

polymorphisms as reliable genetic markers remains to be determined. Further experiments using sequencing data or AFLP data are needed to determine the relationships of the respective *Sargassum* species.

## 6.1 Future Work

Sequencing of genes of algal species facilitates the elucidation of phylogenetic relationship of algae. It is a powerful technique to study the relationships of species especially with the uncertain taxonomic position (Saunders *et al.*, 1995). Lim *et al.*(1983) had already sequenced the 5S ribosomal RNAs (rRNAs) which is 118 base pairs long, from *Sargassum fulvellum*. It is possible to generate molecular probes from the data to identify *Sargassum* samples between closely related species within the genus of *Sargassum*. The 5.8S rRNA gene occurs in the middle region of the ribosomal DNA (rDNA) cistron and is flanked by two internal transcribed spacers (ITSs), ITS1 and ITS2. These spacers contain regions which are highly conserved at the generic level and above, interspersed with highly divergent sequences; large insertions and deletions have resulted in considerable size variation among spacers of different organisms (Appels and Honeycutt, 1986).

Another alternative technique is Amplified Restriction Fragment Polymorphism (AFLP). To solve the problems associated with the taxonomy of *Sargassum* species., AFLP may be a more reliable and stable DNA fingerprinting method to consider as reproducibility of this technique is reportedly very high (Vos *et al.*, 1995). A good correlation between AFLP data and existing taxonomic data will both endow much better descriptions and assessment of biological diversity than those that concentrate on just one approach (Janssen *et al.*, 1996).