1. INTRODUCTION

Archaeological evidence shows that plant cultivation has been in existence since 5000 B.C. (Klug and Cummings, 1991). Domestication of crops and improvement of their productivity have been practiced by humans since the onset of agriculture. Much achievement has been made in this area and genetics has been recognized as the important factor contributing to the successes (Ferh, 1984). Despite the plant breeding progress already achieved, additional gain in agricultural productivity is still needed to cater for the burgeoning growth in world population and changing environment. As a result of rapid growth in world population, global demand for forest products (such as lumber, fiber etc) increases. Such pressure will pass on to the natural forests leading to a need for intensive forest tree plantations with high productivity. Therefore, a large volume of genetically improved planting materials needs to be produced to cater for the demand. Many of the agricultural crops that have a long history of successful breeding programs are usually short generation economic crops such as wheat, maize, barley and rice. Major crops such as wheat and maize have gone through 5 to 10 thousand generations of cultivation and selective breeding (Moffat, 1996). Comparatively, forest trees are very much behind in the genetic improvement programs. In fact the most advanced forest tree breeding programs are only in their third generation (Moffat, 1996). Therefore, in order to achieve higher yield in the area of forestry productivity, new technologies are needed to speed up the forest tree improvement program. The use of molecular marker systems that have emerged over recent years is heralded as one of the technologies with such potential.

1.1 Status of the forestry industry

According to a report by FAO (Food and Agriculture Organization of United Nation) (1995), forests were estimated to cover 27 % of world land area, excluding Antarctica. While 63.7 % of the world's forest were located in the tropics, tropical deforestation worldwide was estimated at 15.4 million hectares (or 0.8 % of the total forested area) annually. In addition, increase in world population will lead to high consumption for forestry products. Annual global demand for forestry products is expected to increase from 3,400,000 m^3 in 1990 to 5,100,000 m^3 by 2010 (Schmincke, 1996; Sedjo, 1996). Natural forests would not be able to supply the projected volume of forestry products. Furthermore, deforestation incurs environmental destruction which could lead to dwindling of biological diversity, upsetting of carbon sequestration and affecting many rural populations whose livelihood depend on the forests. The International Tropical Timber Organization (ITTO) therefore has adopted guidelines for sustainable forest management. In the International Tropical Timber Agreement (ITTA) that was signed in 1984, a target date has been set for all internationally traded tropical timber to be derived from sustainable sources by the year 2000. The policy encourages development of forest plantations as a future supply for forestry products. Worldwide forest plantation is estimated to be at 130 million hectares (Cossalter, 1996). Even though currently tropical plantations represent only a small part of the total world forest plantation, pressure from the demand for forestry products will cause a rapid expansion in this area.

1.2 Status of the forestry industry in Malaysia

Malaysia is the world's largest exporter of tropical logs and sawn timber. It is also the second largest exporter of tropical plywood. According to statistics supplied by FAO (2000), the revenue earned by forest products amounted to approximately US $ 3 billion in 1998. The annual log production in Malaysia for the seventh Malaysian Plan (1996-2000) was estimated to be 28.28 million m^3 (*i.e.* representing 7.37, 5.41 and 15.5 million m^3 respectively for Peninsular Malaysia, Sabah and Sarawak). This figure, however, indicated a decrease of 17% compared to the average of 34 million m^3 under the sixth Malaysian Plan (Chew, 1995). The total annual installed processing capacities of mills in Peninsular Malaysia, Sabah and Sarawak was reported to be 13.2, 7.45 and 6.0 million m^3 respectively (Thai, 1995). Thus, only Sarawak experienced an annual log surplus during the seventh Malaysian Plan period. To cater for the potential increase in demand of downstream processing industry requires rigorous strategy such as establishment of extensive forest plantations.

Tropical rain forest of Malaysia is one of the richest forests in the world. In Peninsular Malaysia alone, there are about 94 woody families from about 760 genera and 4100 species inhabiting the forest (Whitemore, 1972). Nevertheless, most of forest plantations established in the past were from exotic timber species such as

Acacia mangium, *Gmelina arborea*, *Paraserianthes falcataria* and *Eucalyptus camaldulensis* (Rasip *et al.*, 1997). Several reasons were given for greater preference in selecting exotic species over indigenous species. Generally, there is a lack of adequate knowledge in the propagation, silvicultural management and genetic background of indigenous species. In addition to providing a plentiful supply of seeds, the exotic species that are selected for plantation establishment are fast-growing and high yielding. However, there are disadvantages associated with introducing exotic species such as narrow genetic bases and infestation of insect and infection of diseases. Trial plantings have been carried out by Forest Research Institute of Malaysia (FRIM) since 1927 to study the feasibility of using indigenous species for future reforestation programs and plantation establishment. Some potential species (*i.e.* with timber quality and fast growth rate) have been identified which includes *Agathis borneensis*, *Dyera costulata*, *Pentace spp*, *Heritiera spp*, *Azadirachta excelsa* and *Endospermum malaccense* (Hashim *et al.*, 1992). However, currently, knowledge regarding these indigenous species is scanty. Therefore more studies needed to be carried out to introduce these indigenous species into plantation or reforestation programs. *A. excelsa* is selected for this study because it is one of the fastest growing indigenous tree with potential timber quality.

1.3 Status of forest tree improvement

The ultimate aim of forest tree improvement programs is to provide genetically superior stock in replacement for the wild forest stand. The slow progress in forest tree improvement is mainly due to:

1. The long generation time needed for the tree to mature.
2. Low effectiveness of selection for many characters, such as growth vigor, tree form and insect resistance, due to low heritability or difficulty in assessment.
3. The use of open-pollinated seed orchard, since controlled pollination is difficult to perform, which causes the exploitation of only a part of the genetic variation available (Haines, 1994).

Despite the limitations, an estimated 400 tree species have been selected for testing in various breeding programs. Of these, 140 tree species have gone through at least one generation of selection and mating, and 35 of these tree species involved at least 20 seed parents (Committee on Managing Global Genetic Resources 1991). A forest tree breeding programme typically consists of : (a) selection of superior phenotypes (genotypes) from wild populations, (b) replication of selected phenotypes in a clone bank, and (c) recombination of genotypes associated with superior phenotypes through controlled pollination in either a clone bank or seed orchards which have been established in turn from clone banks. Most of the established forest tree breeding programmes comprise of temperate conifer tree species such as *Pinus, Picea* and *Larix* species. In the tropics, even though more than 1000 multi-purpose tree species are cited as being utilised (Cossalter, 1996), more advanced breeding programs only exist in a few species such as Eucalyptus, Acacia, Teak and *Gmelina.* In view of rapid expansion of forestry plantation in the tropics, more breeding programs need to be carried out which incorporate potential forestry species indigenous to the regions. More efficient breeding strategies need to be adopted so that more improved forest tree stock can be obtained. During the past decade, molecular marker technology has shown potential in the application for crop breeding schemes. For example, molecular markers can be used to achieve faster recovery of the recurrent genome in the introgression of desirable genes from a donor genotype. Using tomato as a model plant, Tanksley (1989) showed that total conversion to recipient germplasm in three backcrosses could be achieved through selection with molecular marker screening as compared to a minimum of six for conventional selection. The marker technology detects variations at the DNA level which provides an unlimited amount of genetic variations for analysis. The large amount of genetic variation would be useful for application in many aspects of plant breeding programs such as marker-aided selection, studies on genetic diversity and determination of mating patterns.

1.4 Status of research in *Azadirachta excelsa*

Azadirachta excelsa or Sentang, a member of the *Meliaceae* family, is a tall, fast-growing tree found naturally in lowland tropical forests. A study carried out by the Forestry Department of Peninsular Malaysia (JPSM) indicates that *A. excelsa* is suitable for use as timber, thus it has the potential to be planted as a forest plantation species (Ahmad Zuhaidi and Mohd Noor, 1997). Ng and Tang (1974) found *A. excelsa* to be one of the fastest growing trees in the arboretum of the Forest Research Institute Malaysia (FRIM). A survey on *A. excelsa* trees grown in Southern Thailand shows that the species has an average girth growth rate of 8 – 10 cm per year

(Banasopit *et al.*, 1992). In Peninsular Malaysia, study plots of *A. excelsa* have been established at the Bukit Laggong Forest Reserve, Selangor since 1953 and the Relai Forest Reserve in Kelantan. Some measurements have been made on the standing stock of *A. excelsa* at the Bukit Laggong Forest Reserve in Selangor (Ahmad Zuhaidi and Weinland, 1995 ; Ahmad Zuhaidi and Mohd Noor, 1998). The study showed that the 41 years old standing stock produced a mean annual volume increment of 14.4 $m^3ha^{-1}y^{-1}$. Norwati *et al.* (1997) assessed the genetic diversity of *A. excelsa* from five seed sources collected from different parts of Peninsular Malaysia using isozyme techniques. The isozyme study showed that the average genetic diversity among the five seed sources is low *i.e.* at He = 0.117 (where He is the expected heterozygote frequency). Currently, *A. excelsa* trees have been planted on a wide scale at plantation level in several parts of Peninsular Malaysia and Sabah. The planting stock is mostly from seeds, which would result in a heterogenous population. Unavailability of genetically improved planting stock is the main hurdle facing the plantation of *A. excelsa* at the moment. The genetic improvement work for *A. excelsa* has been slow and genetic information on *A. excelsa* species is generally lacking. With the rapid expansion of forestry plantations, it is imperative that more effort be put in the breeding programs of indigenous trees with timber potential, such as *A. excelsa*, in this region.

1.5 Aims and Objectives of the study

The main aim of this study was to incorporate molecular marker technology into the improvement program of *A. excelsa* in order to obtain genetically improved *A.*

excelsa stock plants to allow the mass production of the plants for plantation purposes. The experimental work consisted of 3 major sections as follows:

1. Establishment of a maternal half-sib population of *A. excelsa* to study the growth pattern of the tree by carrying out periodical phenotypic assessment of quantitative (*i.e.* diameter breast height, total height, number of nodes and canopy diameter) and qualitative (*i.e.* bend/straightness, forking and early branching) traits.
2. Obtain AFLP marker fingerprinting pattern for the study of genetic diversity and mating system of the breeding population. Develop strategy for finding markers linked to desire phenotypic traits in the *A. excelsa* maternal half-sib population.
3. Establish *in vitro* micropropagation protocol for *A. excelsa*. Shoot tips and leaf cuttings from *in vitro A. excelsa* shoot culture were subjected to various phytohormone applications to induce shoot growth and roots formation to form plantlets for the mass multiplication of selected *A. excelsa* plus trees.