Introduction
1. INTRODUCTION

Coconut (*Cocos nucifera* L.) is one of the most useful palms in the world grown in about 93 countries in the tropical belt. Coconut (2n=2x=32) belongs to the family Arecaceae (Palmae), and subfamily Cocoideae, which includes 27 genera and 600 species. *Cocos* is a monotypic genus with no known wild forms. Hence the variability exists only within local types/population. India is one of the leading countries in area as well as production of coconut in the world. The area, production and productivity of coconut in the country are estimated 1.91 million hectares with productivity of 6345 nuts per hectare (http://coconutboard.nic.in). In India, coconut is cultivated in 17 states and three Union Territories. Kerala, Tamil Nadu, Andhra Pradesh and Karnataka are the major coconut producing states in the country, with Kerala alone accounting for 56.5 % of the area and 44.7% of the production.

Coconut is affected by a number of maladies, which adversely affect the production of nuts. Some of them are lethal, while others are of debilitating nature. In the past thirty years, the causes of several coconut diseases of previously uncertain etiology have been determined.
Phytoplasma, virus, viroids and protozoan flagellates have been implicated as the cause of some of these diseases.

The major disease in Kerala is root (wilt) disease of coconut. It is a debilitating disease, which was first reported over a century ago. It is contiguously prevalent in eight Southern districts of Kerala and sparsely in isolated tracts in a few Northern districts of Kerala and in areas of Tamil Nadu adjoining Kerala (Plate 1). The annual loss due to this disease is estimated to be about 968 million nuts (Anon., 1985). There are no control measures to combat the disease. Although integrated management practices have been recommended to increase the productivity of palms in severely affected areas, the permanent solution can be possible only if a resistant/tolerant variety is available. To improve the yield of coconut, thus it is necessary to (i) screen available germplasm for disease resistance/tolerance and other important traits and identify gene(s) responsible, (ii) introgress these gene(s) into high yielding varieties to get disease resistant/tolerant high yielding varieties/hybrids and (iii) large scale propagation of these resistant/tolerant high yielding palms.
Plate 1: Map of Root (wilt) disease affected states of Kerala and Tamil Nadu
At CPCRI, a large collection of coconut germplasm is being maintained and evaluated for resistance to root (wilt) disease. None of the accessions tested so far have been found to be completely resistant to root (wilt) disease. However, some disease free high yielding West Coast Tall (WCT) and Chowghat Green Dwarf (CGD) palms have been located in the heavily diseased areas (‘hot spots’) in four districts of Southern Kerala, viz., Alappuzha, Kollam, Kottayam and Pathanamthitta. These mother palms are being used in breeding programmes since 1988 (Nair et al., 1996).

Considering the long life cycle of coconut palms the task of examining myriad individual palms to identify the presence or absence of marker for resistance is an arduous and time-consuming job. Molecular markers (RFLP, RAPD, AFLP, SSR and DAF) offer numerous advantages over morphological markers traditionally used in plant mapping- they are much faster, more highly discriminating and less costly.

Identification and tagging of genomic region(s) to the expression of resistance/tolerance against the pathogen in these palms will help not only in better understanding of the inheritance of resistance but also future
crop breeding programmes to develop resistant/tolerant palms either by conventional methods or through biotechnological means.

DNA amplification fingerprinting (DAF) is a multiple arbitrary amplicon profiling (MAAP) technique that uses single arbitrary primers as short as 5 nucleotides in length to produce characteristic and highly informative DNA patterns (Caetano-Anollés et al., 1991). DAF uses low stringency amplification conditions so that primers can anneal arbitrarily at multiple sites on each template DNA strand and initiate DNA synthesis. DAF method utilizes very few reagents in few reaction steps. DAF protocol usually involves two major steps: (1) DNA amplification and (2) separation and visualization of amplification products usually done by polyacrylamide gel electrophoresis and silver staining. The silver stained gels can be scanned and images can be stored in electronic files or gels as such dried and stored as record. DAF can be distinguished from other genome scanning technique by the high primer-to-template rations, simplicity, excellent reproducibility and high multiplex ratios. This requires little experimental manipulation and independent of prior knowledge of DNA sequence.
DAF has been used for identity testing phylogenetic relationships, population and pedigree analysis, molecular characterization, high density mapping, tagging useful genes and marker assisted selection (He et al., 1995; Yazdi et al., 1996; Caetano-Anollés et al., 1995, 1999; Shahnejat et al., 1999; Richard-Molard et al., 1999; Assefa et al., 1999)

The present study was undertaken with the following objective

- Standardization of DNA amplification fingerprinting (DAF) protocol
- Screening of primers
- Identifying molecular markers linked to root (wilt) resistance using DNA amplification fingerprinting (DAF) technology.