INTRODUCTION

The life strategies of multicellular plants are strikingly different from animal species. During post embryogenic development, due to the absence of cell migration and the need for continuous organogenesis, plants maintain organ forming cell files called the meristems, which are highly dependent on environmental as well as developmental or hormonal factors. The ontogenic program of a plant is thus highly flexible and linked to the reversibility of the differentiation state of the somatic plant cells. Under extreme conditions, these cells have to change their fate, either to die or divide and dedifferentiate depending on the needs of the system. In general, the developmental program of a plant is much more open to alternative pathways compared to that of animals and this is manifested at the level of cellular differentiation. In addition to the natural \textit{in vivo} forms of embryogenesis, there are three types of embryo development from \textit{in vitro} cultured plant cells namely \textit{in vitro} fertilization (Kranz 1999), microspore embryogenesis (Reynolds 1997) and \textit{in vitro} somatic embryogenesis (Dudits \textit{et al.} 1995).

In angiosperms, development of zygotic embryos from a unicellular fertilized egg occurs through a series of morphologically identical stages beginning with globular to heart, torpedo and cotyledon stages. Although the morphological description of the different stages of zygotic embryogenesis has been extensively studied using microscopy, studies at molecular and biochemical levels has been significantly blocked by the physical inaccessibility. The introduction of somatic embryogenesis thus provides a versatile model system for investigating different biological events occurring during plant embryogenesis (Zimmerman 1993). Since the initial description of somatic embryo induction from carrot cells for 50 years (Steward \textit{et al.} 1958), this unique developmental potential has been recognized as an efficient method for regeneration of whole plants from a somatic cell by a phenomenon of totipotency of cells. In many crop plants, somatic embryogenesis is the main regeneration pathway and has been
regarded as the *in vitro* system of choice with the potential for mass propagation, protoplast work and production of synthetic seeds. The prospects of using somatic embryos as artificial seeds may revolutionize the area of plant propagation, germplasm storage and seed production. When integrated with conventional breeding programmes and molecular and cellular biology techniques, somatic embryogenesis provides a valuable tool for increasing the pace of genetic improvement of commercial crop species via genetic engineering, in particular for tree crop improvement, compared to annual crops. In addition to widening the pool of useful genes, recent years have seen a dramatic increase in the application of genetic engineering which allows the use of molecular farming for the production of a variety of high value recombinant proteins.

The rubber tree (*Hevea brasiliensis*) occupies a prime position worldwide for the production of natural rubber, regarded as nature’s most versatile raw material. Chemically, natural rubber (cis 1, 4 polyisoprene), is a high molecular weight isoprenoid polymer and is valued for its high performance characteristics. Since several centuries, the usage of rubber is tied up with humankind and material mobility. Practically every movement of life requires this fascinating material ranging from the very basic personal articles to today’s transportation including defense and civilian purposes. At the beginning of 20th century, a series of technological developments in processing research revolutionized the uses of rubber and thereby vastly expanded its application. More than 50,000 rubber based products including tyres, engineering components and latex products are now being manufactured all over the world.

From the native environment of Brazil, rubber tree was introduced to South East Asian countries in 1876, by Sir Henry Wickham (Wycherley 1968). This material with a narrow genetic base has served as the base material for the subsequent spread of today’s millions of rubber plantation across Asia and Africa. Presently, rubber is cultivated in over 40 countries with more than 9.5 millions ha. In India, the rubber cultivation marked the beginning of commercial planting in 1902. Now rubber is grown over an area of 5.97 lakh ha with 4.47 lakh ha under tapping and an annual production of 8.02 lakh tonnes (Indian Rubber Statistics 2007). During the last five decades, rubber plantation sector in India has achieved a spectacular growth in area, production and most notably in
productivity. At present, India occupies the 4th position in terms of total production and more significantly attained the first position in productivity. Today, production of natural rubber in India is the highest among major natural rubber producing countries and the country occupies 4th position in production and consumption of natural rubber in the world.

In *Hevea*, hybridization coupled with vegetative propagation and clonal selection is considered as the most important conventional method of genetic improvement. In India, genetic improvements of *Hevea* were initiated during 1954. *Hevea* breeding programmes are aimed to synthesize ideal clones with high production potential combined with desirable secondary attributes like high initial vigor, thick bark having a good latex vessel system, good bark renewal, high growth rate after opening and tolerance to major diseases. In addition to maximum yield, attention has to be paid on producing clones with low incidence of tapping panel dryness. Additionally, we should also take into consideration to produce location specific clones. Clones with early attainability of tapping girth and high initial yields are also preferred in the later phase of exploitation.

Crop improvement of perennial crop in general and rubber in particular, by conventional breeding is, however, very complicated and time consuming as in many other tree crops. The major limitations are its very narrow genetic base, seasonal nature of flowering and low fruit set, lack of fully reliable early selection methods and pronounced interaction of genotype and environment. Furthermore, traditional *Hevea* breeding is extremely difficult due to the long gestation period up to 6-7 years to attain tappable girth of 50 cm and highly heterozygous nature of the tree. The genetic base for the millions of rubber plantations in the east is very narrow, limited to a few seedlings originally collected from a miniscule of the genetic range in Brazil (Schultes 1977). This lack of genetic diversity also leaves the crop highly susceptible to pathogenic attack. In addition to the original narrow genetic base, the vigorous unidirectional selection practiced over the years has further narrowed down the genetic base resulting a slow down in genetic advancement in recent years (Varghese *et al.* 2000). Moreover, the commercially accepted practice of propagation is budgrafting, which involves the grafting of homogenous scions from high yielding trees into unselected rootstocks. However, the rootstocks which are
derived from open pollinated highly heterozygous seedlings cause stock scion interaction leading to intra-clonal variation in field performance. When considering genetic improvement, vegetative propagation is regarded as an important method for achieving higher genetic gain in a shorter time. In *Hevea* breeding, wider adaptation of clonal propagation by bud grafting is also a serious constraint of conventional breeding. In this context, *Hevea* breeders across the world had realized the scope for broadening the narrow genetic base through biotechnological intervention.

Although classical *Hevea* breeding was limited due to certain constraints, over the past few decades, *Hevea* breeders have focused considerable attention in genetic improvements for evolving high yielding clones and have resulted in the release of outstanding clones for commercial planting. Among them, RRII 105 is a very promising clone with wide popularity (Nazeer *et al.* 1986) in the rubber planting sector having a high yield. Recently, a few clones, RRII 414 and RRII 430 having high yield than RRII 105 were released. However, until now, RRII 105 established the viability of natural rubber cultivation in the traditional rubber growing regions with both small and large growers. Most of the high yielding clones, including the clone RRII 105, were highly susceptible to tapping panel dryness, a major physiological disorder seen in the tapping panel of trees characterized by the partial or complete cessation of latex flow. Since 1904, several studies have been focused on the incidence of TPD. Perhaps, these studies have not result any conclusive information neither on the cause nor prescribe any remedial measure to this syndrome. Development of modified clones tolerant to TPD is therefore one of the pre-requisite in genetic improvement programmes. Abnormal leaf fall caused by *Phytophthora* *spp* is the most devastating fungal disease affecting rubber plantations in India. While traditional breeding has resulted in promising clones like RRII 105 with relative tolerance to ALF, fully tolerance to *Phytophthora* has not been developed so far. Further, susceptibility to other diseases like powdery mildew and leaf spot are also concern to the rubber plantations. Thus improving the clone with the specific objective of disease resistance without compromising on yield and productivity is a task of considerable dimension in crop improvement. Nowadays even in traditional areas, drought stress is very common in fields. Besides wide scale planting in the
traditional belts of Kerala and Kanyakumari, rubber cultivation in India is being extended to different environments like the non traditional zones of Karnataka,Maharastra, Orissa, West Bengal and North East states which are exposed to a wide range of abiotic stresses. Therefore, the genetic plasticity conferring the adaptation of rubber trees to diverse agroclimatic and soil conditions deserves more attention. There is no doubt that conventional breeding programmes will lead the rubber trees to increased latex yield by the release of high yielding clones. However, it is quite possible that the rate of rubber biosynthesis within the trees becomes a limiting factor (Arokiaraj et al. 2002). At this point, the prospect of developing rubber trees with enhanced latex yield is quite promising. Additionally, the success of producing transgenic *Hevea* plants will open up exciting possibilities to utilize the transgenic rubber trees as a versatile chemical factory to produce high value proteins where the recombinant proteins can be extracted continuously and nondestructively by tapping the rubber tree. The search for various biotechnological techniques including tissue culture and genetic engineering therefore hold a great potential in crop improvement programmes.

Genetic engineering provides the plant breeders with new tools to complement and supplement sexual hybridization for improvement of existing clones or the creation of totally new germplasm by the insertion of genes coding for useful agronomic traits. This new approach has provided enormous scope to make specific genomic changes in the elite clones in a relatively short period of time without the loss of any of the desired traits of the parental line. This process involves the insertion of well characterized gene(s) into regenerable cells and subsequent recovery of fully fertile plants with the genes integrated into their genome. Although several useful genes conferring resistance against bacterial and fungal are now available, genetic transformation using these genes of interest is still in infancy stage or rather difficult including *Hevea*, due to the lack of a regeneration system. Central to any transgenic technology is, therefore, the availability of a highly efficient reliable *in vitro* plant regeneration system. While an efficient regeneration protocol is essential, micropropagation is important for the transfer of large number of genetically modified plants to the field within a short span of time. Plant regeneration through somatic embryogenesis is
considered as a powerful tool for propagation. Due to the high proliferation potential and low risk of chimeric plant development, somatic embryogenesis is also a desirable component of plant transformation regeneration protocol (Ammirato 1989). The efficacy of such a system for plant production depends on the efficiency of multiplication and conversion rate of somatic embryos. However, efficient and high frequency somatic embryogenesis pathway for the regeneration of a large number of plantlets from *Hevea*, for rubber clone RRII 105 is still lacking. So far, no histological, biochemical and molecular studies were carried out to understand the somatic embryogenesis events in this clone. An attempt on these aspects will thus help us to understand not only the full mastery of somatic embryogenesis, but also enhances the regeneration frequency. In this scenario, realizing the urgent need for an *in vitro* regeneration system via somatic embryogenesis in *Hevea brasiliensis* (clone RRII 105, a popular high yielding Indian clone) and the potential use of this system in genetic improvement programmes, the present study was undertaken with the following objectives:

- To develop a high frequency plant regeneration system through somatic embryogenesis utilizing immature anther as explants
- To optimize various parameters affecting the efficiency of callus induction, somatic embryogenesis and plant regeneration
- To initiate a long term embryogenesis system
- To study the biochemical changes associated with somatic embryogenesis