Biotechnology for the improvement of the tea crop

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Tea is Sri Lankas prime agricultural asset accounting for 2.6 per each of gross domestic production. Among agricultural exports tea continues to be the major foreign exchange earner with 70 per cent the value of agricultural exports and 13 per cent of total export earnings.

Tea, *Commelia sinensis* (L.)O. Kuntze is a beverage tree crop native to south-east Asia and has been introduced into Sri Lanka from India during 1840s. It is a national commodity of Sri Lanka covering over 222,000 ha of the country and providing 12.8% of the export earnings of the countries economy. Sri Lanka is the second largest tea producing country in the world. Out of about 300 million kg produced annually approximately 92 per cent is exported.

Tea is a perennial crop grown in the low, mid and upper elevations in Sri Lanka.

Several authors have shown that the genetic base of cultivated tea is very narrow due to the recurrent use of same parents for the tea breeding programme over the years. The limited diversity of the crop bears with it several risks, including greater genetic vulnerability to pest and disease epidemics, lack of adaptation to climatic change related stresses, lack of genetic variation for specific quality traits reaching performance plateaus and causes conflict with the potential for sustainable genetic improvement in the long term. Breeding strategies in tea depend on its out breed nature, long generation time from seed to flower and its potential for vegetative propagation.

Although clonal selection has helped to increase yield it tends to exploit only the available natural variability which has been low due to the use of primarily a single seed source, Manipuri jat introduced from Assam. As a result the resultant progeny appear to be genetically less diverse than their parents. Since diverse germplasm is a fundamental requirement for crop improvement it has become essential to utilize non-conventional methods for broadening the genetic base. The variants generated through non-conventional methods could then be exploited in conventional breeding programmes to develop superior clones with a combination of desirable agricultural traits.

Micropropagation

Work on the micropropagation of tea was initiated at the Tea Research Institute of Sri Lanka during the latter part of 1984. The main objective was to investigate the possibility of propagation of tea by tissue culture. Work was initially carried out on clones TRI 2025, CY 9 and PK2. Shoot tips 4-5 cm in length were collected in polythene bags and the leaves with the petioles were stripped off as close as possible to the stem without damaging the axillary buds. As many as possible of the tightly furled leaves around the shoot apex were loosened and removed. They were then surface sterilized in chlorox solution and the shoot tips were then trimmed to 10-15mm in length It was virtually impossible to culture the shoot tips without contamination. However the level of contamination was reduced to about 25% using different media. Browning of the explants was a problem to start with but it was overcome by using the mineral salts at half strength and by adjusting the strength of the sterilant and sterilization time.

The shoot tip explants began to grow within one week of culture when the tightly furled leaves started to elongate and unfurl. The axillary bud of the nodal explant began to show signs of growth by the 2nd week (Fig 1). After 2 weeks the explants were transferred to fresh media with full strength mineral salts. The axillary buds of the shoot tips began to grow about the 4th week from explanting.

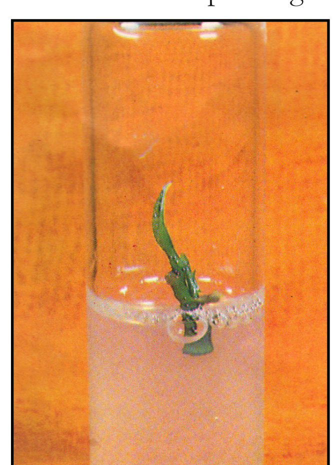

Fig. 1 - Growth of the explant within one week of culture.

Tea responds well to propagation by tissue culture and micropropagation of tea is a distinct possibility in the near future. Using this technique it would be possible to produce hundreds of thousands of clonal plants.

In another study micropropagated shoots of different clones were used to study the effect of auxins (IBA) on micropropagated shoots. The roots formed resembled a tap root system (Fig 4).

In a separate study cotyledon tissue was selected as the explants because it would be free of contamination. Pieces of cotyledon were taken and callus formation was induced and embryoid like structures were formed in 8-12 weeks. The embroids developed shoots and roots within one month of transferring to a medium with less sucrose (Fig.5). After one month in the regeneration medium these plants were successfully acclimatized to plant in the soil.

The present system of vegetative propagation alone is unable to meet the high demand of planting materials. The introduction of new clones takes near by 4-6 years which is the period required to establish the mother bushers for large scale supply. Therefore the use of *in vitro* multiplication technique will be useful in producing a large number of plants within a short period using a few stock plants. The development of a reproducible micropropagation technique for rapid multiplication using various explants is therefore an urgent need. With this primary aim in mind research has been initiated to establish protocols for manipulation of tissues *in vitro*.

In 1986 a method for surface sterilisation for various explants of tea was perfected (modal segments, shoot tips). Differentiated shoot organs as well as tissues (coylodons, stems and leaf tissues) were used as explants to achieve both direct and indirect plant regeneration through different development pathways.

In 1987 research on tissue culture focused on the induction of callus on various explants (cotyledons, leaf and stem) to regerate plants indirethy through the callus phase in colyladom explants. Although embroid structures were found to form in callus initiated from leaf tissue whole plants could not be regenerated.

A protocol for shoot multiplication using rodal explants was patented in 1990.

Efforts were carried out to perfect *in vitro* rooting for the micro shoots from nodal explants. Although the method developed was successful for young seedling explants several limitations were encounterel with clonal material. The major limitations were the contamination with explants, induction of rooting and the long procedure for hardening. At present efforts are being done to scaling up the system for micropropagation.

Haploid Production

Since tea is heterozygous and virtually a self incompatible perennial crop development of homozygous lines through conventional breeding is practically impossible. Therefore studies were undertaken to establish homozygous lines through anther and microspore cultures. In 1997 requirements for initiation of callus from anthers were established. Work is in progress to regenerate plants from anther induced calli.

Manipulation of reproductive cells has a great potential in plant biotechnology. Pollen protoplasts offer a superior source for cell fusion and mutation as they contain a haploid genome. A method for isolating protoplasts from germinating pollen tubes and characterization of the nuclear state of the pollen protoplasts has been established.

Somatic Hybridization

Somatic hybridization through fusion of protoplasms can be used to develop unique hybrids via sexual hybridization. In addition, protoclonal variations which may result in culture could produce novel genotypes with agronomically useful traits without any need for prior hybridization.

Fig. 2 - Growth of the explant and development of axillary buds (one week).

Fig. 3 - Growth of the explant and axillary buds (3-4 week). Note growth of axillary buds.

Fig. 4 - Proliferation of shoots. The large leaves have been trimmed to show axillary shoot development (5-6 week).

An isolated protocol that can release an adequate yield of viable protoplasts from leaf tissue has been accomplished recently. This was developed by optimizing the concentrations and combinations of the enzyme mixture etc.

Fig. 5 - Proliferation of shoots in nodal explants.

As sustained cell division was not observed under the tested culture conditions current work is aimed at refining culture requirements which permit sustained cell division and to regenerate plants.

Wide Hybridization

In the genus *Camellia* post zygotic incompatibilities are very common as the hybrid embryo dies at an early stage of the development in many years owing to endosperm incompatibility. Therefore by growing excised immature embryos *in vitro* it may be possible to rescue hybrid embryos and raise hybrid plants A protocol is now available for culture of embryos and hardening of plamlets raised from immature embryos.

In Vitro **Conservation of Germplasm**

Secure geneic stocks are fundamatal to crop improvement programmes. However the widespread cultivation of a relatively small number of cultivars with the aim of achieving high yield results in loss of diverse genetic materials.
Therefore to reduce the genetic erosion of such germplasm further it is necessary to preserve them for future exploitation. Under the existing *ex-situ* conservation of tea germplasm, establishment and maintenance of tea genetic resources are being carried out only as field gene banks. To further strengthen this *ex-situ* conservation use of *in-vitro* techniques for preservation of plant material is being investigated. In 1998 a preliminary study was undertaken to test the suitability of various explants for preservation under the slow growth method. To increase the rate of survival and revivng efficiency, encapsulation of explants is also being investigated. It is intended to carry out long term *in-vitro* preservation of plant material.

Isozyme Analysis

Conventional breeding methods like selection and hybridization have helped in providing over 60 clones of which 5-7 clones presently occupy over 80 per cent of the clonal extent. This accounts for about 50 per cent of the total Sri Lankan tea acreage. Furthermore of the popular clones 3-4 clones of the TRI 2000 series occupy a large extent. These clones have originated primarily from a single genetic source designated as ASM 4/10 and this has narrowed the genetic base. A detailed genetic analysis of the percentage of commercially cultivated tea based on their isozyme phenotypes, was undertaken in order to confirm the extent of genetic diversity and to check the existing information on perentages. Based on the isozyme on phenotypes of the two parents observed at the G/P locus the 85 clones tested were categorised into seven groups. On observing the isozyme phenotypes of the two parents with the offspring clones 41 out of 46 clones for which both parents were known confirmed the presence of parental bands. The parental analysis further revealed that out of the 46 clones all except four could be traced back to the single ASM 4/10 genotypes thereby proving the narrow genetic base.
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Molecular Markers

Study on isozyme analysis in commercially cultivated tea clones have shown that the genetic diversity of our clones is very narrow. Work with DNA markers would provide an opportunity of allowing a higher level of variability. The results of this project will help determine the genetic identity and determine the genetic diversity between existing tea clones.

Acknowledgement
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