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This special issue of the International Journal of Tea Science on tea breeding and germplasm evaluation has been long in the making. It traverses the span of tea development from the discovery of Assam type plant by Robert Bruce in 1823 to the dawn of 21st century when transgenics are on the horizon. It is organized in two distinct parts. The first part comprises of seven papers dealing with tea breeding and germplasm evaluation for using biotechnological tools. The second part contains >200 scientific abstracts of global research reports on tea, which are classified into 32 subject matter groups, ranging from agronomy, and biotechnology to health effects, tea manufacture, marketing and history & culture.

Overview of the contents

Seven papers dealing with tea breeding and germ plasm evaluation can be roughly divided into four classes. The first two papers by Singh and Apostolides are reviews of tea breeding work done at Tocklai and Malawi since 1930s till date. They are a study in contrast of the access to germplasm variability, the strategies adopted, the techniques employed and the problems faced. The next two papers by Raina *et al.* and Tripathi *et al.* are on biotechnological evaluation of tea germplasm through a study of the chromosomal structure and molecular markers, which are the very basis of modern plant breeding. Next paper by Ahuja and associates describes the techniques of breeding transgenics in tea, using both biolistic and *Agrobacterium* mediated transformation methods. The last two papers by Palni and associates discuss the problems and prospects of tissue culture techniques for rapid mass propagation particularly in reference to good and reliable rooting of selected clones to speed up changeover to improved germplasm in extensive areas under tea plantations and modern methods for testing “true to typeness” or genetic fidelity of micropropagated plants using modern tools as well as physiological, biochemical and other tools.

Tea Breeding in India

In India seeds imported from China were sown for commercial production in early years of 19th century when the tea supplies from China to the Great Britain stopped following the Opium Wars. Assam type plants were taken up for cultivation after their commercial value was proven with good prices realized in 1836 at London Auctions. The local Assam types crossed freely with the plants brought from China and Indochina, giving India an edge in terms of great variability in germplasm collection, which has been a breeders’ delight. I.D. Singh describes how the diverse plant populations were utilized by pioneer tea planters and later by the scientists to develop desirable seed populations and candidate clones. With over 2500 accessions, Tocklai has the largest collection of tea germplasm in the world. The scientists also had access to enormous variations in plant types and plant performance, which is preserved in tea plantations spread over half a million hectares in North Eastern India.

Enterprising tea planters made initial selections of desirable seed material for raising plantations suited to their tea estates. Exploitation of a wide genetic variability in the early years by mass selection gave a number of distinct seed varieties or *jats*. A simple method of vegetative propagation developed by Tunstall in 1931 at Tocklai led to the development of genetically uniform clonal varieties. Informed debates raged on the superiority of highly adaptable (to micro-variations in soil underneath) seed varieties and genetically uniform but rigid (in their optimal needs) clonal plants. Development of polyclonal (similar to composites in corn) or biclonal (double corss hybritds) have the advantage of genetic

adaptability and uniformity within a narrow plant type. Recommendation emerged that seed raised *jats* and clonal varieties should occupy commercial tea plantation in the ratio of 1:1 and that no single clonal variety should be planted in more than 10% area. Breeding for desirable cultivars required alternation of generations between seeds and clonal genotypes for best varietal development programme. In mass selection, which has been the mainstay of varietal work at Tocklai, for every clonal variety 40 to 80 thousand bushes are screened in the field. It was followed by line breeding between selected genotypes. The paper describes procedures and criteria in selecting elite clones, using physical characteristics and simple manual methods for field-testing for 12-15 years before desired clonal variety is released. Conservation of fast depleting, valuable germ plasm present in the existing old plantations is strongly advocated. It assumes greater significance in view of a narrowing genetic base by depending upon a few clonal varieties in breeding programmes, which is fraught with danger of genetic vulnerability.

Tea Breeding in C&S Africa

A review of varietal development work in South & Central Africa by Apostolides and associates traces the history of tea development in Malawi and South Africa since its introduction in 1850. The first commercial plantations of tea with Indian hybrids were in Kearnsey Tea Estate, near Durban in 1877. A flourishing tea industry was established in South Africa by the turn of the 19th century. In a parallel development, Chinese tea seeds were imported in Malawi from Kew gardens in 1876. In the early 1930s Indian Hybrid seeds were imported in Malawi and several other African countries. A unique feature of African tea is a narrow germplasm because of the strict phyto-sanitary embargo on import of new material, which helped in eliminating major pests and diseases. TRFCA established in 1933 proposed a breeding strategy to tackle a hostile climate and breed tea varieties for quality and yield related parameters. Screening methods based on chemical and molecular analysis are being developed by the scientists in Central and South Africa, to reduce the period of varietal selection process and eliminate genotypes with poor quality before the expensive mini manufacture stage.

Comparison of the two Breeding Programmes

Breeding strategies and techniques are a study in contrast between the two groups. North East India had access to wide germplasm variability, plenty of manpower for field selection, good climate, preponderance of pests and diseases, and availability of London and Calcutta based experienced tea tasters for organoleptic tests for quality assessment. In contrast, TRFCA had a narrow genepool, adverse climatic conditions, hardly any pests & diseases, and a demand to breed for specific quality parameters. The services of tea tasters were expensive so the breeders depended heavily on costly biochemical analyses to eliminate poor quality bushes before the mini-manufacture stage and reduce the cost of breeding as well as time span required for developing new tea varieties.

Chromosomal & molecular constitution of tea genomes

An understanding of the chromosomal make up and knowledge of the genetic markers are pre-requisites to launch modern tea breeding programmes. Almost self-sterile and out-breeding nature of the tea plant, coupled with frequent and spontaneous hybridization of different species and genera, resulted in highly heterozygous plants ranging from very small leaf China type to very large leaf Assam type. Some scientists believe that "bevergial tea plant" comprise of many species and genera in their makeup. The hybridization is so extreme that even molecular markers like RAPD and CAPS could not resolve correct identification of cultivated clones.

Chromosomal constitution of tea clones

The cytology of cultivated tea is very poorly understood. Sharma and Raina reported karyotypic analysis of the constitution of 31 Indian varieties of tea clones. Barring one, all the investigated clones were diploids (2n=2x=30) while UPASI-3 was a triploid (2n=3x=45). The clones manifested a chromosomal homeology in chromosomal pairing during meiosis, between the genomes of tea clones, which are known to be "highly heterozygous". It appears that the genomes of the involved taxa are not sufficiently differentiated but exhibit cryptic hybridity, which is sufficient to differentiate clones in their morphology as well as their agronomic traits. The occurrence of trivalents in the only triploid UPASI-3 indicates that it might have originated as segmental allotriploid. The knowledge of genomic constitution is a prerequisite for development of elite tea varieties.

Molecular Markers

With recent focus on tea germplasm conservation, there is an explosion of research on characterization of genetic diversity in the existing accessions. Conventionally, morphological, cytological, biochemical analyses and protein based markers were used to estimate the genetic diversity. But all of them suffer from poor reproducibility. Molecular markers are quite reliable. The paper by Tripathi and colleagues describes various types of molecular markers available and the results of their use in characterizing the genomes available for work on improvement of tea planting material.

Transgenic Tea

Conventional tea breeding has been targeted at selection for yield and quality parameters. More sophisticated breeding techniques are required to breed for resistance to biotic and abiotic stresses, as well as for varieties with specific bioactive constituents, which are suited to provide pharmacological, industrial, cosmetical and nutraceutical products. For incorporating these multigenic characters, transgenic technology appears to be most time-effective method for stacking desirable genes into tea varieties for better adaptation, improved quality and higher crop yields, apart from cultivars suitable for manufacture of high-value products from tea. However, introduction of transgenics in tea faces several problems, including lack of reproducible regeneration systems and resistance of explants and proliferating calli to genetic transformation.

The paper by Ahuja and his colleagues discusses the problems of producing transgenics in tea. Both biolistic and agrobacterium mediated techniques have been used to transform organ explants and somatic embryos. While *Gus* (marker) and *nptII* genes were used to optimize parameters and develop protocols for production of transgenics, plants expressing stress tolerance genes (osmotin) have also been produced. Micro-shoots from the transformed "emblings" (plants obtained from germination of somatic embryos) were excised and further multiplied *in vitro.* Transgenic plants expressing a novel superoxide dimutase (SOD) gene have been produced at IHBT Palampur to reduce winter dormancy in tea. Employing biotechnological tools for producing transgenics with desirable traits is now feasible through isolation of important genes of catechins and flavonoid biosynthesis pathways. Due attention must be paid for the choice of the gene of interest, its sequence, its characteristics, its source, selection of marker genes and expression vector. However, before releasing a transgenic variety, segregation and stability of the selected gene in successive generations on its various traits, must be extensively studied. Field performance trials for tea transgenics have not yet been conducted. Transgenics conforming to the requirements of the industry are on the horizon but more time and effort are needed before they actually ARRIVE, to keep a promise for which the tea industry waits with bated breath.

Tissue culture for mass propagation

Tissue culture technology and mass clonal propagation with advancements in the field of genetic engineering are likely to make a major impact on the tea industry. These techniques include rapid, mass clonal propagation of selected clones, production of pure breeding lines, germplasm storage & exchange, interspecific & intergeneric hybridization, development of haploids, polyploids and mutants, leading to the overall improvement in yield and quality of tea. While several laboratories have successfully developed *in vitro* propagation protocols, the use of modern technology for mass multiplication of tea for commercial plantings is limited largely due to problems of rooting, hardening and survival after field transfer of tissue culture raised plants.

Palni and associates have reported an original research on formation of a well-developed rooting system in micro propagated shoots, which is one of the major constraints in success of any micropropagation protocol. They clearly established that IBA (175 μM) is quite effective in stimulation of rooting in tissue culture propagated microshoots. Temperature and its duration during tissue culture, also determine the success of rhizogenesis and growth of good, enduring root system. Other problems in successful acclimatization of plantlets and their establishment in the field are also alluded to, including the technique of biological hardening that was earlier reported by Pandey *et al.* from the same group of researchers.

Fidelity & Stability of Regenerants

The goal of any *in vitro* propagation system is mass multiplication of plantlets, which should be phenotypically uniform, genetically akin to the mother plant and grow into plant populations that are stable in their performance lasting the whole lifetime of the tea plantation. Nandi and associates have reviewed studies on physiological, biochemical and genetic fidelity of the regenerants, using modern tools of biotechnology. Genetic fidelity was primarily attributed to the genetic make up of the material used for *in vitro* propagation, and not to the culture conditions. Further, regeneration through shoot tip culture is a low risk method for maintaining genetic fidelity. The review emphasizes the need of generating fundamental information on tea plants using physiological and biochemical methods in addition to modern tools of genetic engineering for testing genetic fidelity of tissue culture raised plants. Further it describes the value of such tools for developing strategies for improving the overall growth performance with enhanced quality of tea plants.

Epilogue

Breeding of commercial clonal varieties has served the tea industry well for most part of the 20th century. Paradigm shift in varietal parameters, further complicated by the moving goal posts set by the industry, require tea breeders to develop protocols for efficient gene transfer and effective mass propagation techniques. The promise of genetically engineered tea has been talked about for two decades now but the industry still waits with great expectations for the tea varieties, which are tailor-made for every situation and product profile. Experience with annuals like corn and rice will not be easy to replicate in commercial cultivation of GM (genetically modified) varieties for a long-duration tree crop like tea. Scientifically, there is a felt need to collate information on all aspects of modern breeding techniques in tea and bring it to the attention of all concerned.

I am grateful to the contributors and the guest editor Dr. L.M.S. Palni for this forward looking blue print of the needs of the tea industry in 21st century. The delay in publishing this issue of the IJTS, for reasons beyond the control of the editors, is deeply regretted.

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