Review Article

Chapter 5

TRANSGENIC TEA

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ABSTRACT

Like most of the important crop plants of the world, transgenic technology has also been extended to tea. Both biolistic and *Agrobacterium* mediated transformation methods have been employed to transform explants like leaves and somatic embryos. While *gus* and *npt*II genes were used to optimize parameters and develop protocols for transgenic production, plants expressing stress tolerance genes (osmotin) have also been produced. These methods have opened a whole new era for developing tea plants akin to the 'golden clone' of tea where yield and quality parameters can be combined to conform to the requirements of the tea industry.

INTRODUCTION

In view of the many limitations to horizontal improvement, transgenic technology is the most popular method for vertical crop improvement (Galun and Breiman, 1997). Despite protests from naturalists against genetically modified plants (USDA, 2000), most of the important crop plants of the world have been genetically transformed either through biolistic or *Agrobacteria*. Many of these transgenics have been commercialized and are being routinely used across the globe (James, 2000). Thus, it is not surprising that transgenic technology has also been extended to the most widely consumed and oldest non-alcoholic caffeine-containing health beverage i. e. tea or *Camellia sinensis* (Jankun *et al.*, 1997; Chen, 1999).

IMPORTANCE OF TRANSGENIC PRODUCTION

Tea is becoming increasingly important for its pharmacological properties and its biological constituents viz. flavonoids, amino-acids, vitamins, caffeine and polysaccharides (Chen, 1999). The level of vitamin C in green tea is comparable to that of lemon and liver. Tea besides bio-concentrating fluorine, have anti-caries properties (Chen, 1999) and is known to improve the beneficial intestinal micro-flora. Tea drinking today, is associated with anti-aging, increased cell mediated immune function, immunity against intestinal disorders, cardio-vascular diseases and cancer (Chen, 1999). A recent monograph edited by Jain *et al.* (2006) reports protective effects of tea on a wide range of human diseases, including lifestyle related ailments. Hong (2002) and Hara (2003) have listed several industrial and medicinal uses of tea products and their constituents. An increasingly felt need of the international tea industry is to breed new varieties with specific bioactive constituents for products with important pharmaceutical, industrial, cosmetical and nutraceutical properties.

A major emphasis of the tea industry in India is on increased yield. However, it also has to cater to critical standards which generally vary from region to region. For significant commercial value in the world market, there is also a persistent pressure on the industry for tea varieties that are not only high yielding but also have quality parameters (Jain, 1999). Thus, plants akin to the 'golden clone' of tea are in great demand.

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Tea plants growing in the climatically variable regions also vary in both their yield and quality characteristics (Panda *et al.*, 2003). While the Kangra tea of the western and Darjeeling tea of the eastern Himalayan foothills are valued for their flavour characteristics, the tea growing in Assam and the foothills of Nilgiris (the UPASI cultivars) have larger leaf areas with better adaptability and are thus high yielding varieties. An ever moving goal, that the scientists of each of these regions try to achieve is the breeding of tea plants that are not only high yielding with good cup characters but are also more tolerant to biological and abiological stresses (Wachira, 1990, Jain and Newton, 1990; Bhattacharya and Ahuja, 2004). However, the conventional crop improvement programs cannot cater to these targets fully in the limited available land since tea has certain inherent problems (Mondal *et al.* 2004). Moreover, most of the traits governing the characteristics like tolerance to stresses, yield and quality are multigenic characters and cannot be dealt in a time effective manner with the conventional breeding approaches. Efforts towards increasing yield would also entail combating the losses due to different biotic and abiotic stresses as tea plants are highly susceptible to many of these stresses (Wahab, 2004). In view of all this, transgenics appear to be the most time effective technology for stacking desirable genes into tea for better adaptation, quality and yield (Bhattacharya and Ahuja 2001), irrespective of the limitations of a specific region or a particular product quality.

PROBLEMS EXPERIENCED DURING TRANSGENIC PRODUCTION

Producing transgenic tea with combined characterisitcs of yield and quality is easier said than done. The major limitations to application of transgenic technology to a new crop include problems like lack of a reproducible regeneration system and stubborn resistance of explants and plants to overtures of genetic transformation (Hansen and Wright, 1999). This is largely due to absence of cells competent for both transformation and regeneration. Successful transformation of plants demands that certain criteria be met. The requirements for transformation are (a) target tissues competent for propagation or regeneration, (b) efficient DNA delivery method, (c) agents for selection of transgenic tissues, (d) ability to recover transgenic plants at a reasonable frequency (e) a tight frame in culture to avoid somaclonal variation and last but not the least (f) a simple, efficient, reproducible, genotype independent and cost effective process (Hansen and Wright 1999).

Production of transgenic plants requires cells or tissues that are not only competent for gene transfer and integration but also ones that would regenerate successfully into *in vitro* plants (Chateau *et al.*, 2000). Generally, there is a marked preference for somatic embryogenesis or a proliferating callus from which adventitious shoot buds or somatic embryos can be regenerated in recurrent cycles. This is because gene transfer techniques generally transform only individual or very few cells in an explant. Hence a proliferating callus with a few transformed cells would allow their rapid multiplication leading to the formation of large sectors of transformants (Kapila *et al.*, 1997). Predetermined meristematic tissues like the apical or the axillary buds are generally not preferred (Hewezi *et al.*, 2002) especially in the *Agrobacterium* mediated transformation systems in order to avoid the formation of chimeras or mosaic plants (Riva *et al.*, 1998). Moreover, use of explants from selected elites for transgenic production not only retains the clonal fidelity of the elites but also enables gene stacking. The prime requisite for transgenic production is therefore, a highly reproducible regeneration system *in vitro* (Gelvin, 2000).

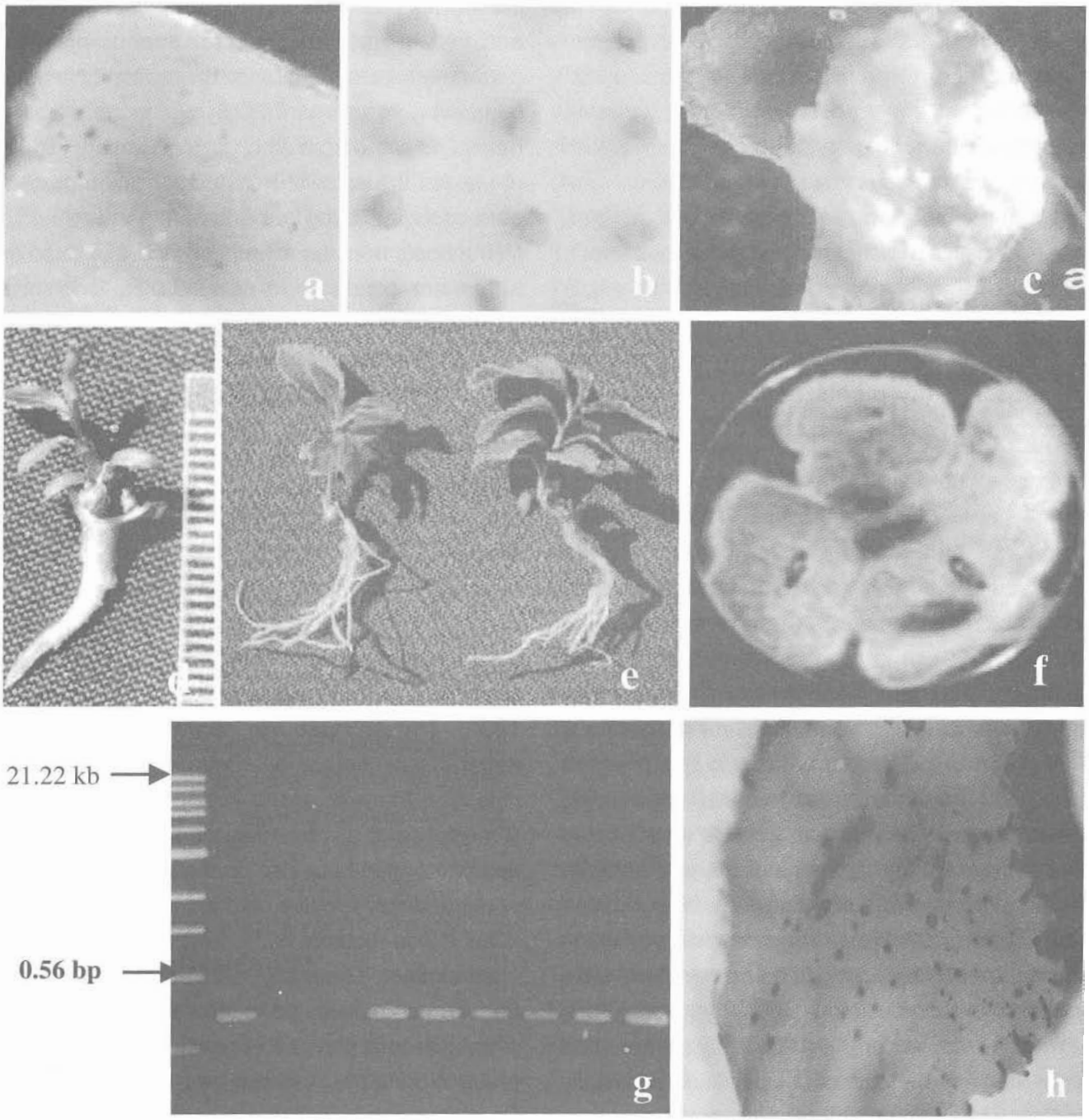
Optimization of the interactions between *Agrobacterium tumefaciens* and plants is another important aspect to be considered and includes parameters like integrity of bacterial strain, its correct manipulation, and interaction of *Agrobcteria* with wounded plant cells. This is because many a times, the tissues may develop necrosis or may affect the release of inducers or repressors of *Agrobacterium* virulence system. Generally, establishment of optimal conditions for gene transfer through preliminary experiments involving transient gene expression using reporter genes is first recommended (Riva *et al.*, 1998). Once optimized, the conditions are then extended to elite genotypes using genes of interest. However, elite genotypes are generally less amenable to *in vitro* culture (Hansen and Wright, 1999). Production of transgenic plants may be extremely difficult for many plants and have also been encountered in different explants of tea.

Factors affecting *Agrobacterium* mediated transformation of tea

Since somatic embryogenesis is one of the most worked out regeneration system in tea (Jain and Newton, 1990), it is not surprising that the first transgenic tea plants were produced from somatic embryos employing *Agrobacterium* harboring a binary vector pBIN19 containing the plasmid p35SGUSINT (Mondal *et al.*, 1999). While developing these transgenics, considerable efforts were directed towards optimization of parameters like explant size, inoculum density, co-cultivation time, co-cultivation medium and the method of *Agrobacterium* infection (Mondal *et al.*, 2001a). These parameters were optimized on the basis of β-glucuronidase (GUS) activity as evident from the blue spots/sectors that appeared after 48 hours of co-cultivation and finally spread to such an extent that the entire embryos turned blue (Fig. 1a, b, c). Pre-culturing of explants and wounding of somatic embryos were found to have no effect on the transformation efficiency. However, bacterial cell density of 10^9 cells/ml corresponding to the log phase of growth (O.D. value = 0.60), 5 days of co-cultivation and a pH of 5.6 (co-cultivation medium) were important for the production of transgenic tea plants. When about 0.5 mm somatic embryos putatively transformed using these parameters were germinated on a selection medium containing kanamycin (200 µg/ml), the emblings (plants obtained from embryo germination; Fig. 1 d, e) yielded the specific PCR amplification products corresponding to the *gus* and *npt*II genes (Fig. 1g). Microshoots from these emblings were excised and further multiplied *in vitro.* About 3.0 cm long shoots with slightly woody nature, were later micro-grafted on to the seedling-derived root-stocks of the same cultivar (Prakash *et al.*, 1999).

Another important aspect that requires extensive manipulation during the production of transgenics is the optimization of correct dose of 'selection antibiotic'. Employment of effective concentrations of selection antibiotic helps in combating a major problem in woody plant transformation i. e. the occurrence of 'escapes' or false positives (Kobayashi and Uchimiya, 1989; Hidaka *et al.*, 1990; Moore *et al.*, 1992). The escapes are non-transformants that escape selection and continue to grow on the selection medium along with the transformants. Occurrence of an 'escape' not only depends upon the marker gene but also on the accurate detection of lethal dose, species, explant and the antibiotics used. It also requires rigorous optimization for each plant/explant. Generally, cross-protection of non-transformed cells by transformed cells or presence of endogenous non specific *npt*II gene are known to result in 'escapes' (Jordan and McHughen, 1988; Dandekar *et al.*, 1988). In order to ensure that only transformed cells persist and grow further to develop

Fig. 1 (a) Somatic embryo showing GUS spots; (b) & (c) complete GUS transient expression in somatic embryos of tea (d)& (e) somatic embryos germinated normally into plants (f) Overgrowing *Agrobacterium* on leaf explants (g) PCR amplification PCR amplification product of gus gene in putative transformants where Lane M is 1 kb ladder, Lanes 2 & 3 are DNA from untransformed plants; Lanes 4-9 are DNA from transformed plants obtained from *Agrobacterium* mediated transformation of somatic embryos (h)GUS transient expression on leaf explant



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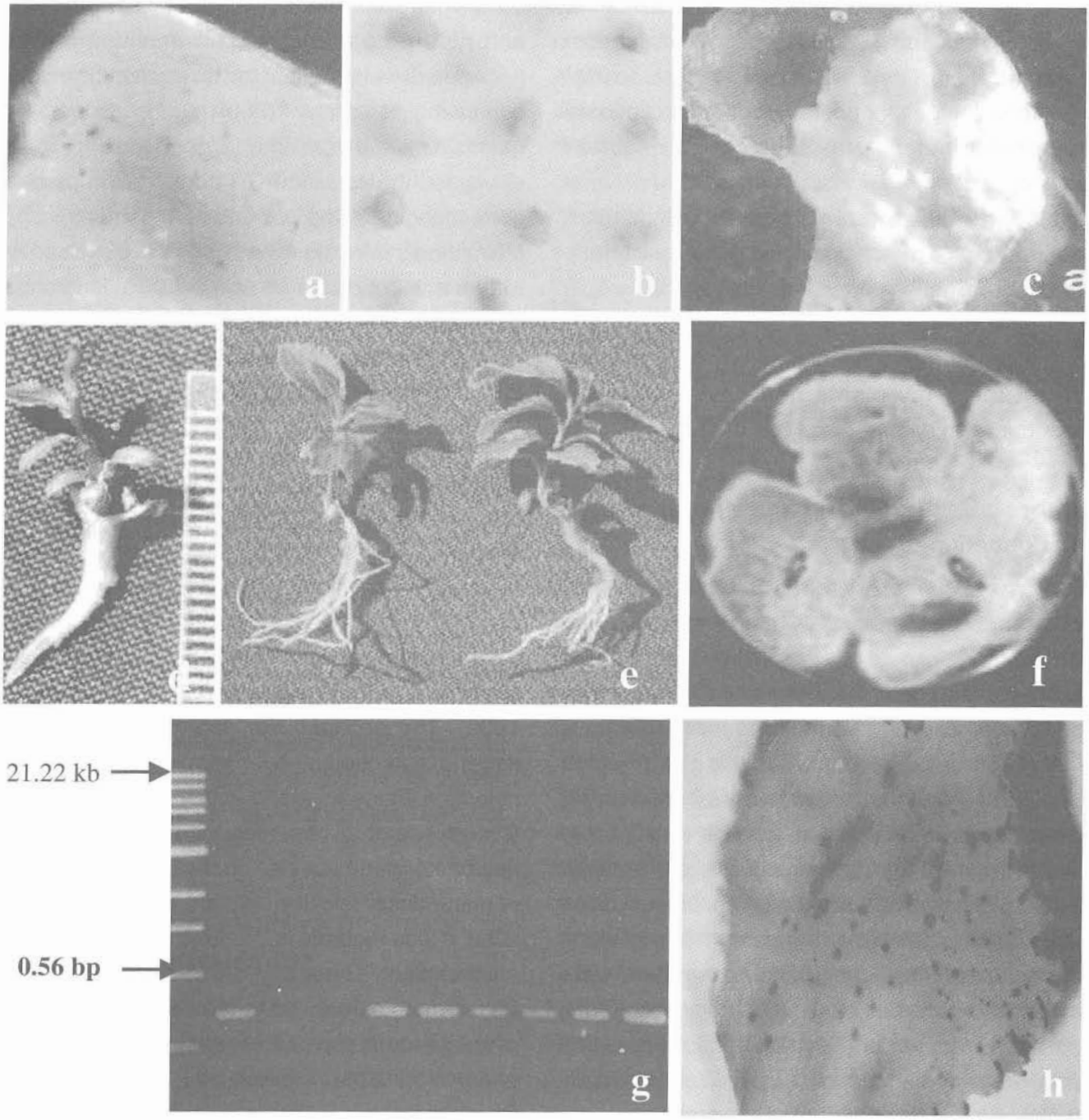


into a plantlet, selectable markers are used in the regeneration medium. These can be divided into several categories depending on whether they confer positive or negative selection and whether selection is conditional or non-conditional based on the presence of external substrates. Positive selection markers are those that promote the growth of transformed tissue whereas, negative ones result in the death of the transformed tissue (Miki and McHugh, 2004). Further, a conditional positive selection system comprises of a gene coding for a protein that confers resistance to a specific substrate that is otherwise toxic to untransformed plant cells. Non-conditional selection systems do not require external substrate yet promote the selective growth and differentiation of transformed material (Miki and McHugh, 2004). While Tosca *et al.* (1996) detected a lethal dose of 75 mg/l kanamycin for selection of internode segments of shoots in some *Camellia* hybrids, Mondal *et al.* (2001b) reported the use of 50 mg/l followed by 75 mg/l kanamycin for the selection of putative transformants (i.e. transformed somatic embryos). However, Sandal (2003) later found that an initial dose of 200 mg/l kanamycin for young shoots (0.5 to 1.0 cm) followed by 500 or 1000 mg/l kanamycin for shoots (3.0 cm or more) eliminated all chances of 'escapes' and yielded high frequency of stable transformants. Other workers like Matsumoto and Fukui (1999) reported the use of 200 mg/l kanamycin for the selection of transformed leaf callus during *Agrobacterium* mediated transformation.

A serious drawback of *Agrobacterium* mediated genetic transformation system is the requirement of a highly cost and labor intensive step of washing the explants after co-cultivation. Washing eliminates the residual *Agrobacterium* that continue to grow and proliferate on the transformed explants (Mondal *et al.*, 2004). Although an important factor on which the success of any transformation experiment is ultimately dependant, yet only a scant systematic effort has been made in this regard (Mondal *et al.*, 2004). As a consequence, a large number of valuable transformants are often lost due to the overgrowth of the residual *Agrobacteria* that may be left on the explant. If not controlled properly after co-cultivation stage, an overgrowing *Agrobacterium* generally produces a mucilage-like compound that completely covers the explants (Fig. 1f) leading to its necrosis and death (Mondal *et al.* 2004) and finally significant reduction in the transformation efficiency of the explants. Being a serious problem in tea, considerable effort has also been directed towards this problem. Mondal *et al.* (2001b) tested three antibiotics for their bactericidal effects and found Sporidex to be effective at 400 mg/l. Sporidex or Ceff 250 is a cephalexin group of antibiotic that like others has the ability to eliminate *Agrobacterium* efficiently with minimal toxicity to most plant tissues (Pollocks *et al.*, 1983; Okkels and Pederson, 1988; Sultana and Ahuja, 1993). Besides being cheaper with easy availability, Sporidex is also known to promote the regeneration potential of different explants (Hewezi *et al.*, 2002) in tissue culture. Hence it is not surprising that Sporidex enhanced the secondary embryogenesis potential in tea significantly (Mondal *et al.*, 2001b). Carbenicillin followed by Cefotaxime appeared to be equally effective in controlling the residual *Agrobacteria* on somatic embryos of tea and hence a combination of Sporidex and Carbenicillin was preferred by Mondal *et al.* (2001b) while developing transgenics from somatic embryos. Although carbenicillin and cefotaxime, both show plant hormone like activity, little is known about the mode of action of these antibiotics. However, a breakdown product of carbenicillin i.e. phenyl lactic acid was reported to exhibit auxin like activity by Holford *et al.* (1992) while working with snapdragon

(*Antrirrhinum majus*) callus. Since an auxin dependent approach was reported to be beneficial both for effective elimination of residual *Agrobacterium* and also regeneration, Tosca *et al.* (1996) used 129 µM cefotaxime. Any increase in the concentration resulted in a decrease in the regeneration ability of *Camellia* x *williamsii*.

While working with leaf explants, Sandal *et al.* (2002 a, b) found that leaves of certain cultivars could not be infected by *Agrobacteria*. This led them to hypothesize that extracts of tea leaves could be a potent bactericidal agent and probably had the potential of controlling the residual *Agrobacteria* from the transformed explants. When used for elimination of *Agrobacteria* after the co-cultivation step, extract of tea leaves completely destroyed the explants, despite being effective as a bactericidal agent. This was due to the oxidation of the high contents of polyphenolic compounds contained in the crude extract completely destroyed the explants. On fractionation, the crude leaf extract yielded catechin and caffeine fractions, which on being used in minute quantities showed diverse activities. While the catechin fraction proved to be an effective bactericidal agent when used at 25, 50, 100, 200 mg/l (Sandal *et al.*, 2002a), the caffeine fraction was highly thermolabile, promoted *Agrobacterium* growth and could be used as a substitute of acetosyringone (Sandal *et al.*, 2002b). Use of microgram quantities of catechin fraction besides being effective in elimination of the residual *Agrobacteria*, circumvented the problem of polyphenol oxidation, thereby allowing the explant to remain healthy as potent transformants.

Gene integration via *Agrobacterium* mediated transformation

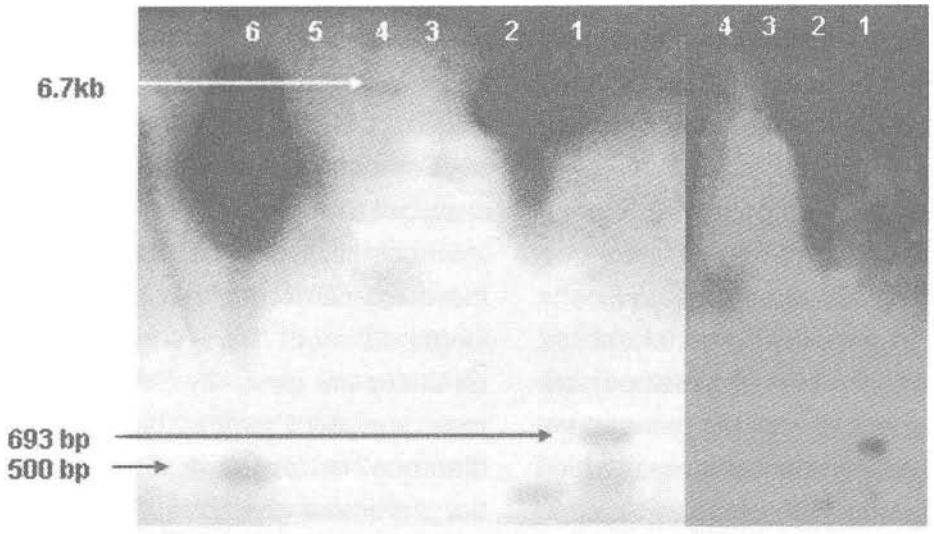
Agrobacterium tumefaciens mediated genetic transformation of tea has also been attempted by several workers and different explants like *in vitro* leaves, stem segments etc. have been employed (Matsumoto and Fukui, 1998, 1999; Biao *et al.*, 1998; Luo and Liang, 2000). However, only putatively transformed leaf callus with stable gene integration was obtained by these workers (Matsumoto and Fukui, 1989, 1999; Biao *et al.*, 1998). While Matsumoto and Fukui (1998, 1999) reported stable transformations in leaf callus after molecular characterization through PCR and southern hybridization, it was Mondal *et al.* (2001) who produced 12 independent kanamycin resistant but GUS positive plants. Of these, only 5 plants showed PCR amplification products of 693 bp and 650 bp corresponding to *npt-II* and gus genes. An internal trangene fragment of 1.6 kb that hybridized to the *npt*II probe (Fig. 2a) was also generated in Southern Blots. Some transgenic lines showed additional short fragments thereby, indicating multiple insertion, rearrangement and/or deletions of the integrated transgenes in the regenerated plants. This is common to *Agrobacterium tumefaciens* mediated transformations which are known to occur during transformation or subsequent regeneration (Svitashev *et al.*, 2000).

Agrobacterium rhizogenes and hairy root formation

Besides *Agrobacterium tumefaciens* mediated genes transfers, attempts have also been made by Zehra *et al.* (1996) towards the development of *Agrobacterium rhizogenes* hairy roots. Thirty five days old *in vitro* leaves were infected with the *A. rhizogenes* strain A4 and explants were transformed with bacterial cell density of 10^8/ml followed by co-cultivation in dark for 2 days. After removal of residual *Agrobacteria*, the leaves were cultured on MS for 35 days for hairy root induction. Stable integration of the transgene was confirmed through paper

Fig. 2: (a) Radioactive labeled Southern hybridization of plants growing in the culture Lab. conditions showing 6.7 kb fragment, after digestion swith EcoR V Restriction enzyme.

(b) Southern blot of plants growing in Greenhouse using DIG labeled probe showing a linear fragment of about 6.7 kb in the independent transformants obtained from biolistic mediated transformation of Kangra jat leaves growing in the greenhouse after digestion with EcoRV.



(a)
Lane 1: A 693 bp fragment of nptII & 490 bp fragment of gus
Lane 2: pRT99gus plasmid DNA digested with Eco RV showing a gus fragment of about 490 bp
Lane 3: DNA from untransformed plant
Lane 4: DNA from transformed plants digested with Eco R1 showing 6.7kb fragment
Lane 5: DNA from untransformed plant
Lane 6: pRT99GUS showing a gus fragment of 490 bp

(b)
Lane 1: lambda DNA digested with Hind III
Lane 2: pRT99gus plasmid DNA digested with Eco RI
Lane 3: untransformed plant DNA
Lane 4: DNA from transformed plants digested with Eco RI showing fragment of 6.7kb

Fig. 2A

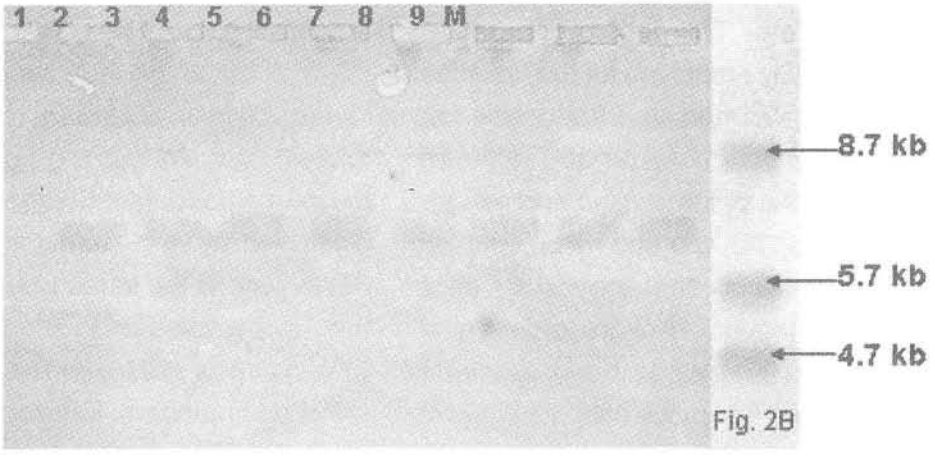


Fig. 2B

electrophoretic analysis of mannopine from the roots. *A. rhizogenes* was also used by Konwar *et al.* (1998) for rooting of 4-6 months old *in vitro* shoots. The basal ends were transformed with *A. rhizogenes* followed by co-cultivation in liquid basal MS medium supplemented with IBA (5 mg l-1) and rifampicin (100 mg l-1). Roots at the basal end of micro-shoots were initiated after 32-45 days of culture in 66% explants and these rooted plants could be hardened in nursery beds.

Biolistic mediated genetic transformation and transgenic production

All earlier attempts to genetically transform tea explants using biolistic as an alternative method have probably failed due to lack of suitable parameters. Thus Akula and Akula (1999) could only report a high transient GUS expression (1085 blue spots/shot) after bombardment of tea somatic embryos with gold particles (1.5-3 µm), coated with plasmid p2k7 DNA. With the advent of an efficient regeneration system wherein adventitious shoot buds could be developed from leaf callus (cultivar Kangra jat) through uptake, metaboiism and depletion of 2,4-D (Sandal *et al.*, 2005) genetic transformation of explants other than somatic embryos became possible. This was a significant achievement in view of the fact that genetic fidelity of elites could be retained using this method as compared to the seed derived somatic embryogenesis system. This leaf regeneration system was successfully employed for the first time for production of biolistic mediated transgenic tea plants (Sandal *et al.* 2002). About 360 different parameter combinations of target, gap and macro-carrier flight distances (TD, GD and MFD) coupled with burst pressure, DNA concentration and osmotica were tested on young leaves of Kangra jat cultivar of tea (Sandal *et al.* 2001). While burst pressure determines the power of the shock or acoustic wave entering the bombardment chamber and may damage the target tissue, the distances are crucial for actual penetration of the microcarriers (gold or tungsten particles) coated with DNA. The effect of the gas or shock wave on microcarrier velocities is determined in part by the 'gap distance' or the gap between the rupture disc and the macrocarrier. Thus, the smaller the distance gap, the more powerful is the effect of gas shock wave on the macrocarrier acceleration. Similarly, the distance between the macrocarrier and the stopping screen is the 'macrocarrier flight distance' and any instability in the macrocarrier flight is known to increase with longer distance'. Thus, shortest macrocarrier flight distances are generally recommended. One of the most important parameters to optimize is 'target distance' or target shelf placement within the bombardment chamber. This placement directly affects the distance that the microcarriers travel have to reach the target tissue for microcarrier penetration and transformation (BioRAD Manual). Based on these principles, a unique set of parameter combination comprising of 1100 burst pressure, target distance of 6 cm, macro-carrier flight distance of 16 mm and a gap distance of 3/8+1/4 was used and about 100% transient GUS expression was obtained (Fig. 1 a, b and c on page 42). For this sorbitol (0.25M for 4 hrs) treated young leaves of tea were bombarded with 1-4 µg of plasmid (pRT99GUS) DNA. Of the total number of putative transformants generated through this method, about 40% survived the lethal dose of kanamycin and yielded PCR amplification products for both *npt*II and *gus* primers were also obtained in these transformants. Southern hybridization further confirmed the stable transformation (Fig. 2b on page 45) in 40% of the kanamycin selected microshoots that were grafted on to seedling-raised-root stocks (Prakash *et al.*, 1999) and hardened in CO_2 and light enriched chambers. Interestingly, the standardized

parameters persistently produced transgenic plants that showed a single copy insertion of the entire linearised plasmid (Fig. 2b) (Sandal, 2003).

Transgenics expressing genes for better adaptability

Tea is a perennial plant with a life span of 60-100 years and is prone to many biotic and abiotic stresses that reduce its yield by more than 50% (Table 1). Of the different biotic stresses, the pests and fungal diseases are worth mentioning. More than a thousand species of arthropod pests are known to attack tea all over the world. However, only 300 species of insects and mites are recorded in India. The pests that attack the tea plants include cockchafers, sucking insects like aphids, mealy bugs, scales, thrips, leaf hoppers and mites; the stem root and wood borers like beetles, termites, caterpillars and a wide variety of caterpillars, ants and locusts that chew up the tender leaves (Sivepalan, 1999). Various pest management programmes are in progress like chemical control, cultural control, escape strategy, trap crops, soil amendments, hybridization and field selections. The age old commercial formulations of *Bacillus thuringiensis,* an efficient bacterium that causes an infectious disease amongst various species of insects has also been used successfully in the control of different species of torticids (*Adoxopheys orana* and *Homona magnanima*) attacking tea (Kariya, 1977). Lately, Tata Tea in collaboration with Bose Institute, Kolkata attempted the production of transgenic plants expressing *Bt* gene. But an important fact that requires mention is any individual strategy in itself is inadequate to effectively and economically manage a pest for long periods of time (Somachaudhury *et al.*,1995) and this has been proved over the years. Tea plants experience severe yield reductions due to lower temperature and high light intensity. Thus there has been a continuous effort towards increasing production through management practices, planting of improved cultivars etc. Adding to the problem is the fact that the crop remains dormant for varying periods in different countries depending upon their

Table 1

Stresses	Causal Oragnism	(%) Yield Loss	Genes that can be employed
Weeds	*Mimosa pudica, Amaranthus spinosus, Solanum khasianum, Ageratum conyzoides*	9	EPSPS (5-enol pyruvyl shikimate-3-phosphate synthase), GST (Glutathione- S-transferase), (Yu *et al.*, 2003) ALS (Acetolactate synthase) (Nifantova *et al.*, 2005)
Insect pests	Lepdopterans and Hemipterans	6-14	Bt Gene, Cowpea trypsin inhibitor (Lingling *et al.*, 2005)
Diseases			
Leaf Diseases	*Exobasidium vexans, Corticium theae, Pestalotia theae, Colletotrichum theae-sinensis*		
Stem Diseases	Tunstallia aculeate, Poria hypobrunnea, Phomopsis theae, Hypoxylon sepens	35	Chitinase, Osmotin (Li *et al.*, 1999) Glucanase, RIP (Ribosomal Inhibitior proteins) (Cho *et al.*, 2001)
Root Diseases	*Ustulina zonata, Hypoxylon asarcodes, Armillaria melloa, Xylaria species*		
Abiotic stresses	Oxidative stress, salinity, drought, cold	10	A novel SOD (Super oxide dismutase) (McKersie *et al.*, 2000) isolated at IHBT, BADH (Betaine aldehyde dehydrogenase (Liang *et al., 1997),* Mannitol 1-phosphate dehydrogenase)

climatic conditions. As a result of which, there is considerable decline in yield. While dormancy occurs for a period of up to six months in countries like Turkey and Iran, dormancy ranges for 2 to 4 months in Argentina and Mauritius. Variations in dormancy are also seen within the Indian sub-continent. Thus, while tea in the north eastern region experiences 2-3 months of dormancy, tea in the Himachal Pradesh in the north western part of the country has an extended dormancy period of 5 to 6 months. Thus, transgenic tea plants expressing a novel super oxide dismutase gene are being produced at IHBT with an aim to impart better tolerance to different climatic stresses and hence to reduce the period of winter dormancy in tea.

Blister blight disease caused by the fungi *Exobasidium vexans* alone has been reported to reduce the yield of tea by about 50% (Jain,1999). Thus the optimized protocol for transgenic tea production using biolistic was also extended to the transfer of osmotin and chitinase genes into somatic embryos and leaves of tea (cv. Kangra jat). Transformed somatic embryos of tea harboring osmotin and chitinase genes were converted into plants and were transferred to containment facility. The plantlets yielded the correct PCR amplification product and showed single gene integration in DIG labeled Southern blots.

Besides the above, improvement of tea has been a major objective of many organizations. Thus, male sterile lines using barnase gene and the fertility restorer gene barstar can be used to eliminate the labor intensive process of manual emasculation for controlled pollination and hybridization in conventional breeding programs. In this regard, IHBT has actually made the application of biotechnological tools for tea improvement feasible through isolation of important genes of catechin and flavonoid biosynthetic pathway for their use in transgenic with improved quality.

Although no reports on the field performance of transgenic tea plants are available till date, the plants growing in the containment facility are bound to see the light of day and will surely be transplanted in the field within a few years. In view of this fact, the regulatory guidelines on Biosafety issues for transgenics have assumed greater importance in tea, especially because cross pollinated plants are considered to be most unsafe as they have the potential to outcross with their wild relatives and pose the maximum danger of gene escape. Being a highly heterozygous and cross pollinated species, the risk of gene escape from tea into related species appears to be quite high. However, this is actually not true. Firstly, the flowering patterns of tea and its wild and related species vary considerably and occur at different times of the year (Wu, 1960). Moreover, the inter-racial crosses in tea is most successful only when Chinary clones are used as female plant and Assamica ones as the pollinators (Bezbaruah and Saikia, 1977). Assamica clones have been reported to be poor as female parent (Bezbaruah, 1977). The risk of a monstrous hybrid being produced is also alleviated by the fact that most inter racial crosses have been reported to be sterile (Singh, 1999). Thus, when tea was used as a female parent, reciprocal crosses with relatives like *Camellia irrawadiensis, C. japonica*, *C. caudata* and *C. kissi,* though successful, failed to produce any seeds. Last but not the least; tea is primarily and mostly cultivated through vegetative propagation only. Employment of vegetative propagation methods is so strictly followed that seed production is restricted only to seed basis of only a few specialized gardens. Thus gene escape from tea is almost nullified.

The risk of transgenic tea plants posing undesirable or damaging effects to human and animal health is also alleviated by the fact that tea leaves either black or green are processed through a series of steps

which involve killing of the leaves and hence denaturation of the transgene product.

CONCLUSIONS

Despite the advantages that support transgenic cultivation in tea, the threats that transgenic generally imposes cannot be ignored. Thus, any programme on transgenic tea production should also include a thorough assessment of the risks involved with the transgenics. Besides, conducting extensive field trials on the extent of gene escape from transgenics and taking adequate measures to contain the plants, care should also be taken with respect to the choice of the 'gene of interest' and its sequence, its characteristics, its source, the selection of marker genes to be used, the characteristics of the plant expression vector and the transformation system that is to be employed. Moreover, genetic analysis on the gene integration, segregation and stability of the gene in successive generations and its effect on the morphology, physiology and biochemistry of the plants and its product should be extensively studied prior to the release of transgenic plants in the open. Once these aspects are clearly worked out producing transgenic tea catering to the demands of the industry and cultivating them for maximum commercial benefits appears to be most effective method for targetted varietal breeding in tea.

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