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Review Article

Applications of molecular markers to the genetic improvement of *Camellia sinensis* L. (tea) – A review

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SUMMARY

In recent years, considerable emphasis has been placed on the development of molecular markers for a variety of objectives. However, the development and application of molecular markers in tea breeding are recent, dating back only to the mid-1990s. This review focusses on the different molecular markers used in the genetic improvement of tea, both locally and internationally. The majority of molecular marker studies in tea have been confined to genetic diversity analysis, while only a few studies have attempted to construct genetic linkage maps to facilitate marker assisted selection (MAS). So far, no reports have appeared on the practical application of MAS in tea breeding programmes, illustrating the fact that there is much work still to be done to make the MAS strategy a practical reality in tea breeding. Analyses of the genetic diversity of tea germplasm using different molecular marker techniques (RAPD, AFLP and ISSR) to date, and other areas for the application of molecular markers in tea, are discussed. The opportunities offered by the integration of molecular markers into conventional tea plant improvement programmes, highlighting the synergy of conventional breeding and MAS for genetic improvement of tea, are reviewed. Future perspectives on the application of molecular markers to various objectives related to the different stages involved in tea crop improvement, to enhance its efficiency and the cost-effectiveness of MAS, are outlined.

Tea (*Camellia sinensis* L.) is one of the high priority plantation crops, which makes a significant contribution to the economy of number of countries. It is widely cultivated in many countries located in Asia and Africa. The tea plant is a woody perennial species, which belongs to family Theaceae, in the section *Thea* (Barua, 1963). The origin of tea is thought to be in regions around the source of the Irrawaddy river, to the south-east of China and Assam in north-east India (Kingdon-Ward, 1950). The origin of cultivated tea is in doubt, because of our inability to trace the ancestry to a true wild population (Kulasegaram, 1978). The majority of cultivated forms of *C. sinensis* L. are diploid, with a chromosome number of 30 (2n = 30) (Morinago *et al.*, 1929; Ahmed and Singh, 1993). There are a few instances where natural triploid (2n = 45) cultivars have been found in cultivation (Kulasegaram, 1978; Banerjee, 1992).

Tea breeding objectives are primarily focussed on high yield, high quality of processed tea, and genetic resistance to major biotic and abiotic stresses prevailing in different tea growing regions. In the past, and at present, new tea cultivars have been developed solely through conventional breeding methods. Due to the predominantly out-crossing nature of the tea plant, and the presence of a strong self-incompatibility mechanism (Rogers, 1975), existing natural seed tea populations are highly heterozygous (Barua, 1963). Therefore, mass selection from existing natural seed tea populations (Singh, 1999), and progenies generated *via* natural pollination or controlled (artificial) hybridisation, have been used to select promising genotypes, followed by vegetative propagation of the elite individuals (Richards, 1966).

Breeding and selection in tea involve several stages: crossing of the selected parents, followed by selection and evaluation of the progeny; processes that require many years of testing and evaluation. The parent clones for each cross are chosen so that the weaknesses of one are matched by the strengths of the other, in the hope that a few of their offspring will have the strength of both parents. Because both parent clones are highly heterozygous, extensive trait segregation is apparent in the population of seedlings (Bezbaruah, 1971). Hence, the selection of plants that contain appropriate combinations of traits from a segregating progeny, to identify the few that may have potential as new cultivars, is a critical component of tea breeding. At the same time, the procedures involved in screening and selection in tea have many limitations and need substantial resources, especially land and labour. Furthermore, the long juvenile phase of the tea crop limits the rate of genetic improvement achievable per year. It is important, therefore, to take steps to accelerate and strengthen conventional tea breeding programmes, to achieve genetic enhancement of the crop in a more integrative and rapid manner.

Recently, applications and developments in the field of agricultural biotechnology have expanded. Many of these developments rely on advances in genomics, and the remarkable achievements of this new science have been transferred and exploited in the field of crop plant breeding (Henry, 1997; Prasanna and Hoisington, 2003; Zhang *et al.*, 2006). Molecular markers and marker-assisted selection (MAS) strategies have been used extensively to strengthen and accelerate conventional plant breeding programmes (Mohan *et al.*, 1997;

Kasha, 1999). As tea is still characterised and selected using environmentally- and ontogenetically-dependent morpho-anotomical traits (Green, 1971), molecular marker techniques offer great promise in improving the efficiency of tea plant breeding programmes.

Molecular markers may be broadly divided into three classes based on their method of detection: (1) hybridisation-based; (2) PCR-based; and (3) DNA sequence-based (Winter and Kahl, 1995; Henry, 1997). The first type of molecular marker used in plant breeding was Restriction Fragment Length Polymorphism (RFLP), which is a co-dominant marker (Tanksley *et al.*, 1989). Although RFLPs have been used since the 1980s in several important crop species (Tanksley *et al.*, 1989; Beckmann and Soller, 1983), there are no reports on their application in tea. The earliest PCR-based markers to be applied extensively in plant breeding were Random Amplified Polymorphic DNAs (RAPDs; William *et al.*, 1990) and they have been the most commonly used marker technique in tea (Wachira *et al.*, 1995; 1997; Kaundun *et al.*, 2000; Chen and Yamaguchi, 2002; Young-Goo *et al.*, 2002; Chen *et al.*, 2005a; Mewan *et al.*, 2005). Later, Amplified Fragment Length Polymorphism (AFLP) (Paul *et al.*, 1997; Wachira *et al.*, 2001; Balasaravanan *et al.*, 2003) and, more recently, Simple Sequence Repeats (SSRs) or microsatellites (Freeman *et al.*, 2004; Yao *et al.*, 2005) have been used in tea to achieve various objectives. Major areas of past and present applications of molecular markers in tea are discussed below.

Genetic diversity analysis and germplasm characterisation

. Much of the genetic characterisation of crop germplasm has had the practical goal of discovering genetically diverse lines for breeding (Bretting and Widrelechner, 1995; Spooner *et al.*, 2005). This is a pre-requisite to increase heterosis in any crop (Karp *et al.*, 1997; Henry, 1997). Genetic variation, or diversity, in plants has been traditionally assessed using morphological traits which are highly influenced by environmental factors or the developmental stage of the plant (Winter and Kahl, 1995). On the other hand, characterisation and genetic diversity estimates based on molecular markers, which allow the direct assessment of variation in genotypes, is a better alternative (Winter and Kahl, 1995; Henry, 1997).

Wachira *et al.* (1995) were the first to characterise tea cultivars using RAPDs, which have been the most widely used technique in tea to estimate the extent of genetic diversity, due to their great speed, low cost, and lack of requirement for radioactive labelling. The molecular marker techniques that have been applied to genetic diversity and/or similarity analyses in tea, and related species, are summarised in Table I.

As with tea, various molecular markers have been applied to horticultural tree crop species (Bretting and Widrelechner, 1995; Hokanson *et al.*, 1998; Yuanwen *et al.*, 2001; Hagidimitriou *et al.*, 2005), primarily to investigate the diversity among genotypes of the cultivated gene pool. On the other hand, some studies aimed to identify diverse genotypes from exotic and unadapted germplasm (Tohme *et al.*, 1996), which is a feasible approach to apply in tea, as many desirable traits such as resistance to biotic and abiotic stresses reside in such germplasm. Furthermore, it would be timely to investigate the genetic diversity of natural, old seedling tea populations that exist in many tea-growing countries, in order to preserve these diverse populations as *in situ* conservation sites, prior to replacing them with genetically more similar modern tea cultivars. Since this entails a large number of samples and resources, it would be appropriate and economical to adopt the method described by Fu (2000) and Young-Goo *et al.* (2002), which involved RAPD analysis using DNA extracted from pooled samples from each population in order to estimate the diversity between populations.

As the accuracy of the results depends largely on the reliability and reproducibility of the marker system employed, multiple molecular markers (RAPDs, AFLPs and ISSRs) were used in some studies to establish

TABLE I

Summary of work on genetic diversity analysis in tea and related wild species using different molecular markers

Technique	Main findings or emphasis	Reference
RAPDs	Estimated genetic diversity among 38 Kenyan cultivars belonging to three tea varieties (Assam, China and Cambod).	Wachira *et al.* (1995)
RAPDs	Estimated genetic diversity among 20 *Camellia* species to establish the affinity between cultivated tea and its wild relatives.	Wachira *et al.* (1997); Chen and Yamaguchi (2002)
RAPDs	Assessed genetic diversity among 27 accessions from Korea, Japan and Taiwan and established affinities based on geographical origin.	Kaundun *et al.* (2000)
RAPDs	Assessment of genetic diversity between 20 Korean tea plantations to reveal the narrow genetic base of seed tea populations.	Young-Goo *et al.* (2002)
RAPDs	Assessed genetic distances between two groups of cultivars; estate selections and cultivars developed in Sri Lanka.	Mewan *et al.* (2005)
RAPDs	Established genetic relationship among 15 well-known traditional Chinese tea cultivars.	Chen *et al.* (2005a)
AFLPs	Estimated genetic diversity among 32 Indian and Kenyan tea genotypes; genotypes were grouped into three main types: Assam, China and Cambod.	Paul *et al.* (1997)
AFLPs	Assessment of genetic diversity among 49 tea cultivars revealed the narrow genetic base of tea cultivated in South India.	Balasaravanan *et al.* (2003)
AFLPs	Confirmed the close relationship between 29 cultivars growing in the Darjeeling area.	Mishra and Sen-Mandi (2004a, b)
AFLPs	Variable groupings in tea and its wild *Camellia* relatives were identified.	Wachira *et al.* (2001)
AFLPs	Assessed genetic diversity between 37 cultivated and native wild tea cultivars in Taiwan and studied the correlation between results generated from RAPDs and ISSRs.	Lai *et al.* (2001)
AFLPs	Distinguished and established the genetic diversity between Assam and China type tea.	Kaundun and Matsumoto (2002)
ISSR	Established the genetic diversity between six Chinese tea cultivars.	Yao *et al.* (2005)

correlations between the results generated using different molecular marker techniques in tea (Wachira *et al.*, 2001; Lai *et al.*, 2001; Devarumath *et al.*, 2002). Some studies confirmed the superiority of AFLP and ISSR markers over RAPDs (Wachira *et al.*, 2001; Devarumath *et al.*, 2002), while others proved that there was a highly significant correlation between the results generated from RAPDs and ISSRs (Lai *et al.*, 2001). Comparable results were also generated in comparative studies conducted on other crop species (Hagidimitriou *et al.*, 2005; Pejic *et al.*, 1998; Russell *et al.*, 1997). Hence, deciding which technique would be most appropriate for a particular investigation is not always straightforward; it depends on a range of factors, including the molecular nature of the problem, the biology of the species, and the availability of resources (Karp *et al.*, 1997, Henry, 1997).

Marker-assisted germplasm acquisition and formation of core collections

Gene banks are reservoirs of genetic diversity. However, maintenance of maximum genetic diversity is achieved only at a significant cost, especially when a large tree species such as tea is to be conserved as a living collection. The perennial and highly allogamous nature of tea prevents seeds from being used as material for conservation. To facilitate proper utilisation and managerial aspects, core collections have been developed in gene banks following the concept developed by Frankel (1984). It has been confirmed that molecular marker technologies can play a role in supporting traditional germplasm management approaches (Brown, 1989; Bretting and Widrelechner, 1995).

Many tea-growing countries have abandoned seed tea populations, and propose to uproot and replace them with improved high yielding cultivars. However, such populations consist of genotypes that possess traits such as resistance to biotic and abiotic stresses that may not be present in the cultivated gene pool. Hence, it is important to conserve the unique accessions found in these populations, to expand the collections in *ex-situ* gene banks before losing them forever. In achieving this task, it is important to avoid duplicates. It would therefore be important to introduce marker-assisted acquisition and formation of core collections, which has not yet been reported for tea, but has been used for many other crops, with success (Treuren *et al.*, 2006; Virik *et al.*, 1995; Hokanson *et al.*, 1998).

One problem that often occurs in deciding which germplasm should be acquired to expand the diversity of a plant genetic resource collection, and which accessions should be included in a core collection, is the lack of proper data (Spooner *et al.*, 2005). Molecular data on genetic diversity may provide essential information to develop core collections (Hodgkin *et al.*, 1995; Karp *et al.*, 1997; Spooner *et al.*, 2005). Some core collections have been established exclusively based on molecular marker data (Ghislain *et al.*, 1999; Marita *et al.*, 2000), while some were based on a combination of data such as molecular markers and pedigree data (Treuren *et al.*, 2006). Molecular markers should, however, complement characterisation based on morphological or biochemical descriptions, providing more accurate and detailed information when making decisions on new acquisitions, as well as forming core collections (Spooner *et al.*, 2005). Hence, such a coherent approach would be appropriate for making a core collection in tea, as has been successfully adopted for some tropical crop plants (Hamon *et al.*, 1998; Ronning and Schnell, 1994). Furthermore, many genetic diversity studies reported so far in tea have used only a small number of accessions, which did not facilitate the formation of a core collection. Therefore, it is important to include all accessions conserved in gene banks to generate base information for the above purpose.

Phylogenetic analysis

Although various molecular marker techniques have been used for phylogenetic investigations in many other tree crop species (Anthony *et al.*, 2002), to establish relationships between wild and cultivated forms (Arias and Rieseberg, 1994), and to trace ancestries (Koopman *et al.*, 2001), these have not yet been studied extensively in tea. RAPD markers have been used to resolve problems relating to the phylogeny of tea and related species (Chen and Yamaguchi, 2002), and to taxonomic relationships among teas classified under Assam, Cambod and China varieties (Wachira *et al.*, 1995). The results of these studies confirmed the present taxonomy and the known pedigrees. However, it is important to discover the relationships between various tea taxa in order to introgress economically important traits into proven cultivars through classical breeding methods, and to create greater variability for efficient selection. This would also facilitate identifying close relatives of non-tea, *Camellia* species, through phylogenetic studies for inter-specific hybridisation.

Fingerprinting of tea cultivars

"Fingerprinting" is an attempt to discover cultivar-specific molecular markers that can aid their identification (Spooner *et al.*, 2005; Henry, 1997). In general, gene bank collections may consist of multiple accessions of the same cultivar and their identification or discrimination by means of morphological traits would be difficult. Furthermore, improved cultivars are frequently morphologically similar and difficult to discriminate using morphological traits alone. This is particularly true in tea (Wickramaratne, 1981). Although plant genotyping using molecular techniques is one of the most promising avenues, which has immediate practical application and has been used successfully for cultivar identification in some, mainly horticultural, tree crops (Koller *et al.*, 1993; Olivera *et al.*, 1999; Yuanwen *et al.*, 2001), it has been employed very rarely in tea. The usefulness of RAPD markers for fingerprinting Chinese tea germplasm (Chen *et al.*, 2005a), and of AFLPs (Paul *et al.*, 1997) and ISSRs (Yao *et al.*, 2005) for discriminating between tea cultivars which cannot be distinguished on the basis of their morphological traits, have been reported. The superiority of ISSRs over RAPDs and AFLPs has been demonstrated in studies by Lai *et al.* (2001) and by Devarumath *et al.* (2002) who used very closely-related tea cultivars for identification, and micropropagated plants to evaluate genetic fidelity, respectively. In grapes, for example, very closely-related cultivars have been discriminated using ISSRs (Vignani *et al.*, 1996) and ISSRs are a logical choice for a crop like

tea, as commercially grown cultivars are derived from a very narrow genetic base. Being a vegetatively propagated crop further aggravates clonal identification of tea grown in different environments and, hence, the best choice of marker for fingerprinting of tea cultivars would be ISSRs.

It is essential to identify suitable fingerprinting techniques to resolve problems of mis-identification in germplasm, and certification of vegetatively propagated materials to control quality and to protect plant variety rights. These will become integral components of tea breeding programmes in future. In this context, ISSRs will have an important role due to the fact that they are robust compared to RAPDs, technically less-demanding than AFLPs, and iess complex than SSRs.

Hybridity and parentage analysis

Molecular markers are very useful for confirming the hybridity of artificial hybrids, both sexual and somatic (Spooner *et al.*, 2005). RAPDs and AFLPs have been used to resolve such problems, especially when hybridity was questioned for morphological reasons (Parani *et al.*, 1997), and/or for early screening of large putative hybrid populations (Dubreuil *et al.*, 1996). Korzun *et al.* (1997) used SSRs to confirm the genetic constitution of hybrid banana. However, these aspects have not been explored extensively in tea. There has been only one study to confirm the undisputed parental identity of TRF 1, a high-yielding tea clone recently released by the United Planters Association of South India (UPASI), using RAPDs (Balasaravanan *et al.*, 2001). However, there is scope to employ molecular markers to confirm the parentage of new progenies generated by controlled hybridisation as there is a greater chance for contamination of pollen due to human error. Early screening of a putative hybrid population would therefore facilitate eliminating uncontrolled out-crosses, thus saving resources in subsequent phases of evaluation, which is a critical component in tea breeding.

Construction of genetic linkage maps

The process of constructing linkage maps and conducting analysis to identify genomic regions associated with quantitative traits is known as quantitative trait loci (QTL) mapping (also 'genetic', 'gene' or 'genome' mapping; Weeden *et al.*, 1994; Mohan *et al.*, 1997). Linkage maps can be constructed to locate genes of economic importance. A number of extensive reviews exist on this subject (Young, 1994; Haley and Andersson, 1997).

The choice of parents and crosses for use in constructing linkage map is critical, and the size of the mapping population will determine the ultimate resolution of the map (Staub and Serquen, 1996; Henry, 1997). Most linkage maps in plants have been obtained from segregating populations derived from crosses between inbred lines (Staub and Serquen, 1996). However, in tea, being a highly heterozygous and allogamous crop species, the possibility of having inbred lines is very remote. The high degree of variation and heterozygosity found in tea suggests that many markers could be present in the heterozygous state, and therefore could potentially segregate in the F_1 population, allowing the construction of a linkage map. Hence, the approach now widely used to construct genetic linkage maps for highly heterozygous species is the "pseudo-testcross" strategy (Hemmat *et al.*, 1994; Grattapaglia and Sederoff, 1994; Kenis and Keulemans, 2005; Debener and Mattiesch, 1999; Yamamoto *et al.*, 2002). This has been the procedure adopted to construct the two linkage maps available for tea (Hackett *et al.*, 2000; Huang *et al.*, 2005). Hackett *et al.* (2000) were the pioneers in constructing a genetic linkage map for tea. In that study, they used a mapping population that consisted of 90 genotypes which was generated by a cross between two non-inbred tea clones, based on RAPD and AFLP markers. The map constructed by Huang *et al.* (2005) was based on an improved AFLP method. They also used the F_1 progeny of known cultivars as a mapping population which consisted of 69 genotypes. The map included 16 linkage groups and located 200 markers, covering a total map length of 2,545.3 cM.

These maps may provide a starting point for MAS in tea. However, larger populations are required for high-resolution mapping (Mohan *et al.*, 1997; Henry, 1997). Hence, the size of the mapping population used to construct maps for tea seem to be inadequate, because the parents are highly heterozygous. Hence, segregation analysis requires larger numbers of progeny to increase reliability (Henry, 1997). Thus, a mapping population that consisted of 300 genotypes, a segregating population of a cross between two known Sri Lankan tea cultivars, was developed by the Tea Research Institute of Sri Lanka and established in the field (Gunasekare *et al.*, 2003) to facilitate construction of a more accurate molecular map. Grattapaglia and Sederoff (1994) emphasised that the "pseudo-testcross" strategy is efficient at the intra-specific level, and increasingly so with crosses of genetically divergent individuals from geographically distinct origins. Hence, in establishing the Sri Lankan mapping population, consideration was focussed mainly on the distinction between the parents used to derive the mapping population. The parents involved were of entirely different origins: one of Indo-Chinese ancestry, and the other of Indian origin. The same population is now being used for bulk segregant analysis (BSA) to identify genomic regions responsible for tolerance to blister blight disease, as the two parents used to create the segregating population were susceptible and tolerant to the disease (Gunasekare *et al.*, 2003). BSA using extreme phenotypes (typically the upper and lower 5%) is a rapid and powerful tool to identify the number of major loci controlling the trait, although this procedure overestimates the effects of the identified loci (Michelmore *et al.*, 1991).

Concern regarding the true descendants certainly contributes to the quality of the genetic linkage map. However, it is not always possible to obtain the correct progeny through the "pseudo-testcross" approach, since an uncontrolled cross or even self-pollination is likely to occur under natural conditions. Hence, the potential of the "pseudo-testcross" strategy can best be explored if the entire mapping population is screened to identify the true descendants of the two parents, and eliminate individuals in the progeny arising from uncontrolled out-crosses or self-pollination, before subjecting them to marker analysis.

Resistance to biotic and abiotic stresses is a major

objective of tea breeding programmes. Classical methods have been used to develop cultivar resistance to several major diseases and to drought. However, the level of success has been limited due to the low efficiency of breeding. Hence, it is necessary to tag markers linked to these important traits to facilitate future MAS. The lack of reliable screening methods for pest and disease resistance is a serious limitation when developing molecular markers linked to these traits in many crops (Mehlenbacher, 1995), including tea. For some pathogens, it may be difficult to provide conditions for uniform infection for precise screening; nevertheless, such conditions must be provided in order to identify marker loci. Hence, unless otherwise accurate scoring methods can be developed for phenotyping disease resistance, the identification of molecular markers linked to these traits will lead to false information. Under present circumstances, the development of molecular markers linked to such traits, even those governed by one or a few genes is still debatable for tea.

Genetic linkage maps for different tree crop species are currently available, and are being further saturated by dominant and co-dominant markers to obtain higher quality maps (Hemmat *et al.*, 2003). To achieve the ultimate goal of genetic improvement of tea, further efforts are required to construct a high density map using molecular markers, and to integrate economically important traits onto the linkage map. A start has been made by constructing two frame-work maps for tea (Hackett *et al.*, 2000; Huang *et al.*, 2005), and concerted efforts are needed to locate as many markers as possible, linked to important traits. However, as many economically important traits are under complex control by a number of genes, it is imperative to construct high density linkage maps that can be used to dissect QTLs (Zhang *et al.*, 2006). Accurate QTL analysis, and comparative QTL mapping, as with apple (Liebhard *et al.*, 2003), would be a feasible possibility in tea.

Markers such as RAPDs, AFLPs and SSRs are only genetically-linked to the trait of interest, and no functional relationship can be inferred. The latest trends are to combine QTL mapping with functional genomics methods to study gene expression (Stuber *et al.*, 1999). These techniques include expressed sequence tags (ESTs) and micro-array analysis, which can be used to develop markers from the genes themselves (Stuber *et al.*, 1999; Gupta *et al.*, 2001). This offers more promise in identifying the actual genes that control the desired traits (Cato *et al.*, 2001).

Initial studies on the construction of cDNA libraries for tea (Li *et al.*, 2004; Chen *et al.*, 2005 b, c), and analyses of ESTs from these cDNA libraries have been reported (Park *et al.*, 2004; Chen *et al.*, 2005b, c). Data-mining for SSRs in ESTs, and the development of EST-SSR markers also appeared recently (Jin *et al.*, 2006). Furthermore, the numbers of ESTs and genomic sequences available in databases are growing rapidly, especially from international genome sequencing projects. Accumulation of this information will be extremely useful for the discovery of single nucleotide polymorphism (SNPs) and data-mining for new markers in the future (Gupta *et al.*, 2001; Rafalski, 2002). This is also true for the construction of genetic maps and QTL analysis in tea.

Marker assisted selection (MAS)

MAS offers great opportunities for improved efficiency and effectiveness in the selection of plant genotypes with desirable combinations of traits. This approach relies upon the establishment of a linkage between the molecular marker(s) and the characteristic(s) to be selected. Hence, DNA markers that are tightly linked to agronomically important genes (called "gene tagging") may be used as molecular tools for MAS (Ribaut and Hoisington, 1998). This is extremely valuable if the phenotype is difficult or expensive to score (Young *et al.*, 1992); for example, some diseases lead to false assessments due to the lack of adequate or reliable inoculua under natural field conditions. Hence, DNA markers closely linked to such traits would contribute greatly to practical tea crop improvement programmes.

Molecular marker maps, the pre-requisite for any MAS programme, have been constructed for some agriculturally important species (Hemmat *et al.*, 2003; Liebhard *et al.*, 2003; Kenis and Keulemans, 2005). However, the density of these maps varies considerably between species (Ribaut and Hoisington, 1998; Young, 1999; Collard *et al.*, 2005). The predictive value of the genetic markers used in MAS depends on their inherent reproducibility (Weeden *et al.*, 1992; 1994), map position, and linkage with economically important traits (Henry, 1997). The presence of a tight linkage (< 10 cM) between the trait and a genetic marker(s) may be useful in MAS, to increase the benefit from selection (Staub and Serquen, 1996). The average distance between markers in the map constructed for tea by Huang *et al.*, (2005) was 12.8 cM. Therefore, greater saturation would be needed for practical application.

There is considerable divergence between different crop species in relation to the application of MAS (Weeden *et al.*, 1994). Progress in cereals is advanced (Prasanna and Hoisington, 2003; Zhang *et al.*, 2006) compared to most horticultural crop species (Collard *et al.*, 2005), where the development of molecular markers maps has been slow and few are available. MAS may be particularly useful for horticultural crop plants with a long juvenile period.

Appropriate uses and cost-effectiveness of MAS

Once molecular "tags" have been identified for resistance genes, selection based on these tags become possible. Currently, the cost of using molecular markers is the most important factor that limits the implementation of MAS (Stuber *et al.*, 1999). Hence, feasibility and cost-effectiveness also must be considered, particularly when the use of these tools involves a shift of resources away from traditional breeding, rather than the use of additional resources. Furthermore, MAS must always compete, in cost and speed, with alternatives for direct screening for the desired phenotype, to be worthwhile.

Therefore, in future, molecular markers must be user-friendly, accurate, precise, low cost and amenable to high-throughput, potentially automated analysis. The ability to screen for additional markers, at little additional cost, should also be a major consideration (Collard *et al.*, 2005). If MAS can be justified for one or more traits, then the sample-handling, DNA extraction and data handling

costs for the samples may be covered; hence, screening for additional markers or traits may cost very little extra.

The extent to which MAS will be used in practice depends on the relative cost and expected return compared to conventional methods (Dudley, 1993). Its application will, at least initially, be limited to simply-inherited traits that are difficult or expensive to measure or screen. Applications such as: (1) identifying seedlings likely to be resistant [either in the absence of the pathogen, or when reliable conventional screening methods are difficult or expensive (Weeden *et al.*, 1994)]; and (2) constructing pyramids of resistance genes without the need for progeny testing (when multiple races of the pathogen exist; Staub and Serquen, 1996), appear most appropriate for tea. Also it is essential, to ensure cost-effectiveness, that the markers identified would be useful in several crosses, over several generations, rather than being restricted to the progeny of a single cross, already evaluated (Collard *et al.*, 2005). Hence, routine application of MAS requires careful analysis of the relative costs involved in marker development, and the benefits of MAS against conventional screening and selection.

Future dimensions of molecular markers in tea breeding

A wide array of molecular marker technologies are available, and many are increasingly being applied to complement traditional approaches in plant breeding and germplasm management. However, the development and application of molecular markers in tea are still confined mainly to genetic diversity analysis, illustrating the fact that much work remains to be done. To compete in the international tea market, tea breeders' demands should become more focussed on public health concerns and environmentally-friendly production systems. Hence, future tea breeding strategies should be targeted at incorporating multiple pest and disease resistances, as well as developing adapted tea cultivars with a low caffeine content. Many of these important traits reside in wild relatives and allied species of tea. Some low-caffeine, non-tea species are available in World tea germplasm collections; species such as *C. irrawadiensis*, *C. taliensis* and *C. lutenscens*, which are devoid of caffeine (Singh, 1999). Hence, inter-specific hybridisation, followed by molecular breeding, facilitated by marker-aided introgression to incorporate the desired exotic alleles into the adapted genetic background, would be an appropriate strategy to adopt in tea. This would also facilitate broadening the base of available germplasm, which is crucial in a crop like tea that claims to have a very narrow genetic base.

Yield, arguably the most important trait, is polygenic in nature, difficult to measure and/or improve and, highly influenced by the environment. Genomic maps have been constructed for rice (Hittalmani *et al.*, 2002) and *Brassica* spp. (Mahmood *et al.*, 2005) to analyse the stability of detected QTLs, especially those for yield and yield-related traits in different environments. Hence, it would be worthwhile conducting similar investigations to explore yield stability in tea, which is currently difficult to achieve due to strong environmental influences and lack of accurate selection criteria on yield and yield components in tea.

Although there have been numerous QTL mapping studies for a wide range of traits in diverse crop species, relatively few markers have actually been implemented in plant breeding programmes (Collard *et al.*, 2005). In many cases, this may be attributable to a low accuracy of QTL mapping, or inadequate validation (Young, 1999). As statistical methodology is a vital component of mapping studies, it is important to improve mapping software using statistically more powerful methods, not only to facilitate molecular breeding and MAS in tea, but for other crops as well. Furthermore, MAS necessitates using larger population sizes, more accurate phenotypic data, different genetic backgrounds, and independent validation or verification in order to develop reliable markers (Weeden *et al.*, 1994), to prove its practical value.

As discussed, molecular markers have already proved their potential as tools to aid breeding programmes in several important agricultural crops. The same can be expected in tea, in time to come, if the various molecular techniques are optimised to strengthen different activities in tea breeding programmes in a more integrative manner. There is every reason to believe that the synergy of empirical tea breeding, molecular markers, MAS, and genomics will produce greater effects than the sum of the various individual applications and will contribute towards effective tea breeding programmes around the World, to help ensure the sustainability of the tea industry. Ideally these molecular techniques should supplement conventional breeding programmes aimed at developing elite tea cultivars possessing low caffeine contents, greater yield stability over diverse environments, and genetic resistance to major biotic and abiotic stresses, thus producing new tea varieties that thrive better under changing and/or different agro-ecologies, and meet the ever-changing demands of consumers.

REFERENCES

AHMED, N. and SINGH, I. D. (1993). A technique for rapid identification of ploidy level in tea. *Two and a Bud*, **40**, 31–33.

ANTHONY, F. COMBES, M. C., ASTORGA, C., BERTRAND, B., GRAZIOSI, G. and LASHERMES, P. (2002). The origin of cultivated *Coffea arabica* L. varieties revealed by AFLP and SSR markers. *Theoretical and Applied Genetics*, **104**, 894–900.

ARIAS, D. M. and RIESEBERG, L. H. (1994). Gene flow between cultivated and wild sunflowers. *Theoretical and Applied Genetics*, **89**, 655–660.

BALASARAVANAN, T., PIUS, P. K. and KUMAR, R. (2001). Parentage of TRF-1 revealed. *United Planters Association of South India (UPASI) Newsletter*, **11**, 1.

BALASARAVANAN, T., PIUS, P. K., KUMAR, R. R., MURALEEDHARAN, N. and SHASANY, A. K. (2003). Genetic diversity among south Indian tea germplasm using AFLP markers. *Plant Science*, **165**, 365–372.

BANERJEE, B. (1992). Selection and breeding of tea. In: *Tea: Cultivation to Consumption*. (Willson, K.C. and Clifford, M. N., Eds.). Chapman and Hall, London, UK. 26–86.

BARUA, P. K. (1963). Classification of the tea plant. *Two and a Bud*, **10**, 3–11.

BECKMANN, J. S. and SOLLER, M. (1983). RFLPs in genetic improvements: methodologies, mapping and costs. *Theoretical and Applied Genetics*, **67**, 35–43.

BEZBARUAH, H. P. (1971). Tea breeding: A review. *Two and a Bud*, **22,** 123–130.

BRETTING, P. K. and WIDRLECHNER, M. P. (1995). Genetic markers and horticultural germplasm management. *HortScience*, **30,** 1349–1356.

BROWN, A. H. D. (1989). Core collections: a practical approach to genetic resources management. *Genome*, **31,** 818–824.

CATO, S., GARDNER, R., KENT, J. and RICHARDSON, T. (2001). A rapid PCR based method for genetically mapping ESTs. *Theoretical and Applied Genetics*, **102,** 296–306.

CHEN, L. and YAMAGUCHI, S. (2002) Genetic diversity and phylogeny of tea plant and its related species and varieties in the section *Thea*, genus *Camellia* determined by randomly amplified polymorphic DNA analysis. *Journal of Horticultural Science & Biotechnology*, **77,** 729–732.

CHEN, L., GAO, Q., CHEN, D. and XU, C. (2005a). The use of RAPD markers for detecting genetic diversity, relationship and molecular identification of Chinese elite tea genetic resources preserved in tea germplasm repository. *Biodiversity and Conservation*, **14,** 1433–1444.

CHEN, L., ZHAO, L. and GAO, Q. (2005b). Sequencing of cDNA clones and analysis of the ESTs properties of young tea plant (*Camellia sinensis*) shoots. *Journal of Agricultural Biotechnology*, **13,** 21–25.

CHEN, L., ZHAO, L. and GAO, Q. (2005c). Generation and analysis of ESTs from the tender shoots cDNA library of tea plant (*Camellia sinensis*). *Plant Science*, **168,** 359–363.

COLLARD, B. C. Y., JAHUFER, M. Z. Z., BROUWER, J. B. and PANG, E. C. K. (2005). An introduction to markers, QTL mapping and markers-assisted selection for crop improvements: The basic concept. *Euphytica*, **142,** 169–196.

DEBENER, T. and MATTIESCH, L. (1999). Construction of a genetic linkage map for rose using RAPD and AFLP markers. *Theoretical and Applied Genetics*, **99,** 891–899.

DEVARUMATH, R., NANDY, S., RAVI, V., MARIMUTHU, S., MURALEEDHARAN, N. and RAINA, S. (2002). RAPD, ISSR and RFLP fingerprints as useful markers to estimate genetic integrity of micropropagated plants of three diploid and triploid elite tea clones representing *Camellia sinensis* (China type) and *C. assamica*, ssp. *assamica* (Assam-India type). *Plant Cell Reports*, **21,** 166–173.

DUBREUIL, P., DUFOUR, P., KREJCI, E., CAUSSE, M., VIENNE, D. and CHARCOSSET, A. (1996). Organisation of RFLP diversity among inbred lines of maize representing the most significant heterotic groups. *Crop Science*, **36,** 790–799.

DUDLEY, J. W. (1993). Molecular markers in plant improvement: Manipulation of genes affecting quantitative traits. *Crop Science*, **33,** 660–668.

FRANKEL, O. H. (1984). Genetic perspectives on germplasm conservation. In: *Genetic Manipulation: Impact on Man and Society* (Arber, W., Illmensee, K., Peacock, W.J. and Starlinger., Eds.). Cambridge University Press, Cambridge, UK. 161–170.

FREEMAN, S., WEST, J., JAMES, C., LEA, V. and MAYES, S. (2004). Isolation and characterisation of highly polymorphic microsatellites in tea (*Camellia sinensis*). *Molecular Ecology Notes*, **4,** 324–326.

FU, Y. B. (2000). Effectiveness of bulking procedures in measuring population-pair wise similarity with dominant and co-dominant genetic markers. *Theoretical and Applied Genetics*, **100,** 1284–1289.

GHISLAIN, M., ZHANG, D., FAJARDO, D., HUAMAN, Z. and HIJMANS, R. J. (1999). Marker-assisted sampling of the cultivated Andean potato collection using RAPDs. *Genetic Resources and Crop Evolution*, **46,** 547–555.

GRATTAPAGLIA, D. and SEDEROFF, R. (1994). Genetic linkage maps of *Eucalyptus grandis* and *Eucalyptus urophylla* using a pseudo-testcross mapping strategy and RAPD markers. *Genetics*, **137,** 1121–1137.

GREEN M. J. (1971). An evaluation of some criteria used in selecting large yielding tea clones. *Journal of Agricultural Science*, **76,** 43–156.

GUNASEKARE, M. T. K., RANATUNGA, M. A. B. and KOTTAWA ARACHCHI, J. D. (2003). Plant Breeding Report: Establishing seedling population for the identification of molecular markers. *Annual Report of Tea Research Institute of Sri Lanka for Year 2003*, 138 pp.

GUPTA, P. K., ROY, J. K. and PRASAD, M. (2001). Single nucleotide polymorphism: A new paradigm for molecular marker technology and DNA polymorphism detection with emphasis on their use in plants. *Current Science*, **80,** 524–535.

HACKETT, C. A., WACHIRA, F. N., PAUL, S., POWELL, W. and WAUGH, R. (2000). Construction of a genetic linkage map for *Camellia sinensis* L. (tea). *Heredity*, **85,** 346–355.

HAGIDIMITRIOU, M., KATSIOTIS, A., MENEXES, G., PONTIKIS, C. and LOUKAS, M. (2005). Genetic diversity of major Greek olive cultivars using molecular markers (AFLPs and RAPDs). *Journal of the American Society for Horticultural Science*, **130,** 211–217.

HALEY, C. and ANDERSSON, A. (1997). Linkage mapping of quantitative trait loci in plants and animals. In: *Genome Mapping – A Practical Approach*. (Dear, P., Ed). Oxford University Press, New York, USA. 49–71.

HAMON, S., DUSSERT, S., NOIROT, M., ANTHONY, F. and HODGKING, T. (1998). Core collections – accomplishments and challenges. *Plant Breeding Abstracts*, **65,** 1125–1133.

HEMMAT, M., WEEDEN, N. F., MANGANARIS, A. G. and LAWSON, D. M. (1994). Molecular marker linkage map for apple. *Journal of Heredity*, **85,** 4–11.

HEMMAT, M., WEEDEN, N. F. and BROWN, S. K. (2003) Mapping and evaluation of *Malus* × *domestica* microsatellites in apple. *Journal of American Society of Horticultural Science*, **128,** 515–520.

HENRY, R. J. (1997). *Practical Applications of Plant Molecular Biology*. Chapman and Hall, London, UK. 57–133.

HITTALMANI, S., SHAHIDHAR, H. E., SIDHU, J. S. and KHUSH, G. S. (2002). Molecular mapping of quantitative trait loci for plant growth, yield and yield related traits across diverse locations in a doubled haploid rice population. *Euphytica*, **125,** 207–214.

HODGKIN, T., BROWN, A. H. D. and MORALES, E.V. (1995). *Core Collections of Plant Genetic Resources*. John Wiley and Sons, Chichester, UK. 10–32.

HOKANSON, S. C., SZEWC-MCFADDEN, A. K., LAMBOY, W. F. and MCFERSON, J. R. (1998). Microsatellite (SSR) markers reveal genetic identities, genetic diversity and relationships in a *Malus* × *domestica* core subset collection. *Theoretical and Applied Genetics*, **97,** 671–683.

HUANG, J., LI, J., HUANG, Y., LUO, J., GONG, Z. and LIU, Z. (2005). Construction of AFLP molecular marker linkage map in tea plant. *Journal of Tea Science*, **25,** 7–15.

JIN, J., CUI, H., CHEN, W., LU, M., YAO, Y., XIN, Y. and GONG, X. (2006). Data mining for SSRs in ESTs and development of EST-SSR marker in tea plant (*Camellia sinensis*). *Journal of Tea Science*, **26,** 17–23.

KARP, A., KRESOVICH, S., BHAT, K. V., AYAD, W. G. and HODGKIN, T. (1997). Molecular tools in plant genetic resources conservation: a guide to the technology. *IPGRI Technical Bulletin*, **No. 2,** 9–42.

KASHA, K. J. (1999). Biotechnology and world food supply. *Genome*, **42,** 642–645.

KAUNDUN, S. S., ZHYVOLOUP, A. and PARK, Y. (2000). Evaluation of the genetic diversity among elite tea accessions using RAPD markers. *Euphytica*, **115,** 7–16.

KAUNDUN, S. S. and MATSUMOTO, S. (2002). Heterologous nuclear and chloroplast microsatellite amplification and variation in tea. *Camellia sinensis*. *Genome*, **45,** 1041–1048.

KENIS, K. and KEULEMANS, J. (2005). Genetic linkage maps of two apple cultivars (*Malus* × *domestica* Borkh.) based on AFLP and microsatellite markers. *Molecular Breeding*, **15,** 205–219.

KINGDON-WARD, F. (1950). Does wild tea exist? *Nature*, **4191,** 297–299.

KOLLER, B., LEHMANN, A., MCDERMOTT, J. M. and GESSLER, C. (1993). Identification of apple cultivars using RAPD markers. *Theoretical and Applied Genetics*, **85,** 901–904.

KOOPMAN, W. M., ZEVENBERGEN, M. J. and BERG, R. G. (2001). Species relationships in *Lactuca* (Asteraceae) inferred from AFLP fingerprints. *American Journal of Botany*, **88,** 1881–1887.

KORZUN, V., BOERNER, A., WORLAND, A. J., LAW, C. N. and ROEDER, M. S. (1997). Applications of microsatellite markers to distinguish inter-varietal chromosome substitution lines in wheat. *Euphytica*, **95,** 149–155.

KULASEGARAM, S. (1978). Progress in tea breeding. *Tropical Agriculture Research Series*, **No. 11,** 151–160.

LAI, J., YANG, W. and HSIAO, J. (2001). An assessment of genetic relationship in cultivated tea clones and native wild tea in Taiwan using RAPD and ISSR markers. *Botanical Bulletin of Academia Sinica*, **42,** 93–100.

LI, Y., JIANG, C., YANG, S. and YU, Y. (2004). Beta-glucosidase cDNA cloning in the tea and its prokaryotic expression. *Journal of Agricultural Biotechnology*, **12,** 625–629.

LIEBHARD, R., KELLERHALS, M., PFAMMATTER, W., JERTMINI, M. and GESSLER, C. (2003). Mapping quantitative physiological traits in apple (*Malus* × *domestica* Borkh). *Plant Molecular Biology*, **52,** 511–526.

MAHAMOOD, T., RAHMAN, M. H., STRINGAM, G. R., YEH, F. and GOOD, A. (2005). Molecular markers for yield components in *Brassica juncia* – do these assist in breeding for high seed yield. *Euphytica*, **144,** 157–167.

MARITA, J. M., RODRIGUEZ, J. M. and NIENHUIS, J. M. (2000). Development of an algorithm identifying maximally diverse core collection. *Genetic Resources and Crop Evolution*, **47,** 515–526.

MEHLENBACHER, S. A. (1995). Classical and molecular approaches to breeding fruit and nut crops for disease resistance. *HortScience*, **30,** 466–477.

MEWAN, K. M., LIYANAGE, A. C., EVERARD, J. M., GUNASEKARE, M. T. K. and KARUNANAYAKA, E. (2005). Studying genetic relationship among tea accessions in Sri Lanka using RAPD. *Sri Lanka Journal of Tea Science*, **70,** 42–53.

MICHELMORE, R. W., PARAN, I. and KESSIL, R. V. (1991). Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect major genes in specific genomic regions by using segregating populations. *Proceedings of the National Academy of Science of the USA*, **8,** 9828–9832.

MISHRA, R. K. and SEN-MANDI, S. (2004a). Genetic diversity estimates for Darjeeling tea clones based on AFLP markers. *Journal of Tea Science*, **24,** 86–92.

MISHRA, R. K. and SEN-MANDI, S. (2004b). Molecular profiling and development of DNA marker associated with drought tolerance in tea clones growing in Darjeeling. *Current Science*, **87,** 60–66.

MOHAN, M. S., NAIR, S., BAGHWAT, A., KRISHNA, T.G., YANO, M., BHATIA, C. R. and SASAKI, T. (1997). Genome mapping, molecular markers and marker-assisted selection in crop plants. *Molecular Breeding*, **3,** 87–103.

MORINAGO, T., FUKUSHIMA, E., KANO, T., MARUYAMA, Y. and YAMASAKI, Y. (1929). Chromosome numbers in cultivated plants. *Botanical Magazine Tokyo*, **43,** 569–594.

OLIVERA, C. M., MOTA, M., MONTE-CORVO, L., GOULAO, L. and SILVA, D.M. (1999). Molecular typing of *Pyrus* based on RAPD markers. *Scientia Horticulturae*, **79,** 163–174.

PARANI, M., SINGH, K. N., RAGASWAMY, S. and RAMALINGAM, R.S. (1997). Identification of *Sesamum alatum* × *Sesamum indicum* hybrid using protein, isozyme and RAPD markers. *Indian Journal of Genetics and Plant Breeding*, **57,** 381–388.

PARK, J. S. KIM, J. B. and HAHN, B. S. (2004). EST analyses of genes involved in secondary metabolism in *Camellia sinensis* (tea) using suppression subtractive hybridization. *Plant Science*, **166,** 953–961.

PAUL, S., WACHIRA, F. N., POWELL, W. and WAUGH, R. (1997). Diversity and genetic differentiation among population of Indian and Kenyan tea revealed by AFLP markers. *Theoretical and Applied Genetics*, **94,** 255–263.

PEJIC, I., MORGANTE, M., KOZUMPLIK, V., TARAMINO, G. and MOTTO, M. (1998). Comparative analyses of genetic similarity among maize inbreds detected by RFLPs, RAPDs, SSRs and AFLPs. *Theoretical and Applied Genetics*, **97,** 1248–1255.

PRASANNA, B. M. and HOISINGTON, D. (2003). Molecular breeding for maize improvement: An overview. *Indian Journal of Biotechnology*, **2,** 85–98.

RAFALSKI, J. A. (2002) . Novel genetic mapping tools in plants: SNPs and LD-based approaches. *Plant Science*, **162,** 329–333.

RIBAUT, J. M. and HOISINGTON, D. (1998). Marker-assisted selection: New tools and strategies. *Trends in Plant Science*, **3,** 236–239.

RICHARDS, A. V. (1966). The breeding, selection and propagation of tea. *The Tea Quarterly*, **37,** 154–160.

ROGERS, S. (1975). Observations on pollen tube compatibility in tea clones. *Tea Quarterly*, **45,** 91–100.

RONNING, C. M. and SCHNELL, R. J. S. (1994). Allozyme diversity in a germplasm collection of *Theobroma cacao* L.. *Journal of Heredity*, **85,** 291–295.

RUSSELL, J. R., FULLER, J. D., MACAULAY, M., HATZ, B. G., JAHOOR, A., POWELL, W. and WAUGH, R. (1997). Direct comparison of levels of genetic variation among barley accessions detected by AFLPs, SSRs and RAPDs. *Theoretical and Applied Genetics*, **95,** 714–722.

SINGH, I. D. (1999). Plant improvement. In: *Global Advances in Tea Science*. (Jain, N.K., Ed.). Aravali Books International Ltd., New Delhi, India. 427–448.

STAUB, J. E. and SERQUEN, F. X. (1996). Genetic markers, map construction and their application in plant breeding. *HortScience*, **31,** 729–740.

SPOONER, D., TREUREN, R. and VICENTE, M. C. (2005). Molecular markers for genebank management. *IPGRI Technical Bulletin*, **No. 10,** 56–73.

STUBER, C. W., POLACCO, M. and SENIOR, M. L. (1999). Synergy of empirical breeding, marker-assisted selection, and genomics to increase crop yield potential. *Crop Science*, **39,** 1571–1583.

TANKSLEY, S. D., YOUNG, N. D., PATERSON, A. H. and BONIERBALE, M. W. (1989). RFLP mapping in plant breeding: new tools for an old science. *Biotechnology*, **7,** 264.

TOHME, J., GONZALEZ, D. O., BEEBE, S. and DUQUE, M. C. (1996). AFLP analyses of gene pools of a wild bean core collection. *Crop Science*, **36,** 1375–1384.

TREUREN, R., TCHOUDINOVA, I., SOEST, L. J. M. and HINTUM, J. L. (2006). Marker-assisted acquisition and core collection formation: a case study in barley using AFLPs and pedigree data. *Genetic Resources and Crop Evolution*, **53,** 43–52.

VIGNANI, R., BOWERS, J. E. and MEREDITH, C. P. L. (1996). Microsatellite DNA polymorphism analyses of clones of *Vitis vinifera* ' Sangiovese'. *Science and Horticulture*, **65,** 163–169.

VIRIK, P. S., NEWBURY, H. J., JACKSON, M. T. and FORD-LLOYD, B. V. (1995). The identification of duplicate accessions within the rice germplasm collection using RAPD analysis. *Theoretical and Applied Genetics*. **90,** 1049–1055.

WACHIRA, F. N., WAUGH, R., HACKETT, C. A. and POWELL, W. (1995). Detection of genetic diversity of tea (*Camellia sinensis* L.) using RAPD markers. *Genome*, **38,** 201–210.

WACHIRA, F. N., POWELL, W. and WAUGH, R. (1997). An assessment of genetic diversity among *Camellia sinensis* L. (cultivated tea) and its wild relatives based on RAPDs and organelle-specific STS. *Heredity*, **78,** 603–611.

WACHIRA, F. N., TANAKA, J. and TAKEDA, Y. (2001). Genetic variation and differentiation of tea (*Camellia sinensis*) germplasm revealed by RAPD and AFLP variation. *Journal of Horticultural Science & Biotechnology*, **76,** 557–563.

WEEDEN, N., TIMMERMAN, M., HERMMAT, M., KEEN, B. E. and LODHI, M. A. (1992). Inheritance and reliability of RAPD markers. *Proceedings of Symposium on Applications of RAPD Technology to Plant Breeding*. Nov. 1992. Minneapolis, MN, USA, 12–17.

WEEDEN, N., TIMMERMAN, G. and LU, J. (1994). Identifying and mapping genes of economic significance. *Euphytica*, **73,** 191–198.

WICKRAMARATNE, M. R. T. (1981). Variation in some leaf characteristics in tea and their use in the identification of clones. *Tea Quarterly*, **50,** 183–189.

WILLIAMS, J. G. K., KUBELIK, A. R., LIVAK, K.J., RAFALSKI, J. A. and TINGEY, S. V. (1990). DNA polymorphism amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research*, **18,** 6531–6535.

WINTER, P. and KAHL, G. (1995). Molecular marker technologies for plant improvement. *World Journal of Microbiology and Biochemistry*, **11,** 438–448.

YAMAMOTO, T., KIMURA, T., SHODA, M., IMAI, T., SAITO, T., SAWAMURA, Y., KOTOBUKI, K., HAYASHI, T. and MATSUTA, N. (2002). Genetic linkage maps constructed by using an inter-specific cross between Japanese and European pear. *Theoretical and Applied Genetics*, **106,** 9–18.

YAO, M., HUANG, H., YU, J. and CHEN, L. (2005). Analysis on applicability of ISSR in molecular identification and relationship investigation of tea cultivars. *Journal of Tea Science*, **25,** 153–157.

YOUNG, N. D. (1994). Constructing a plant genetic linkage map with DNA markers, In: *DNA Based Markers in Plants*. (Ronald, K. V. and Phillips, L., Eds.). Kluwer, Dordrecht, The Netherlands. 39–57.

YOUNG, N. D. (1999). A cautiously optimistic vision for marker-assisted breeding. *Molecular Breeding*, **5,** 505–510.

YOUNG, N. D., KUMAR, L., MENANCIO, D., DANESH, D., TALEKAR, N. A., SHANMUGASUNDARUM, S. and KIM, D. H. (1992). RFLP mapping of a major bruchid resistance gene in mungbean. *Theoretical and Applied Genetics*, **84,** 839–844.

YOUNG-GOO, P., KAUNDUN, S. S. and ZHYVOLOUP, A. (2002). Use of bulked genomic DNA-based RAPD methodology to asses the genetic diversity among abandoned Korean tea plantations. *Genetic Resources and Crop Evolution*, **49,** 1–7.

YUANWEN, T., TANABE, K., TAMURA, F. and ITAI, A. (2001). Genetic relationship of pear cultivars in Xinjiang, China, as measured by RAPD markers. *Journal of Horticultural Science & Biotechnology*, **76,** 771–779.

ZHANG, Y., MIAN, A. R. and BOUTON, J. H. (2006). Recent molecular and genomic studies on stress tolerance of forage and turf grass. *Crop Science*, **46,** 497–511.