INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 6, No. 1, p. 318-325, 2015

RESEARCH PAPER OPEN ACCESS

Molecular analysis by RAPD markers of popular tea (*Camellia sinensis*) varieties of North-East India infested by tea mosquito bug (*Helopeltis theivora*)

Shaheen Shah[1*], RNS Yadav[1], PK Borua[2]

[1]*Centre for Studies in Biotechnology, Dibrugarh University, Assam, India*

[2]*Department of Life Sciences, Dibrugarh University, Assam, India*

Key words: *Camellia sinensis*, RAPD, *Helopeltis theivora*, molecular variation, polymorphic information content, discriminatory power.

http://dx.doi.org/10.12692/ijb/6.1.318-325 Article published on January 10, 2015

Abstract

Tea (*Camellia sinensis*) drink is prepared in North-East India from four popular tea clones viz; TV1,TV23, S3A3 and Tinali. The research conducted to study the molecular variation among these tea clones in relation to *Helopeltis theivora* infestation. A total of 27 genotypes of tea plants(26 infected and 1 healthy sample) consisting of 4 varieties -TV1, TV23 (most susceptible)S3A3,Tinali(moderately susceptible)was collected from 7 leading tea gardens located in Dibrugarh district, Assam, India. RAPD was performed using 10 random primers. A total of 113 bands were found with 90 polymorphic bands and 23 monomorphic bands in all 27 accessions. The polymorphism % of the bands indicated considerable genetic variation (78.82%)with average banding pattern of 16.9. The discriminatory power 'D' ranged from 0.92 to 0.98 for 9 primers(A-I) except primer J(0.81). While the polymorphic information content of 4 primers ranged from 0.25 to 0.35(medium informative locus). Genetic similarity estimated by Jaccard's similarity coefficient showed high variability (average coefficient value 0.52%).The dendogram constructed by UPGMA method generated 3 clusters with majority of Tinali,S3A3 and TV1 samples in clusters A,B and C respectively. TV23 accession indicates genetic similarity with TV1 which might explain the reason for their higher susceptibility. While S3A3 accession showed similarity with Tinali group which explains lesser susceptibility. Thus the RAPD results showed high polymorphism among the varieties and revealed that plants with lower infection might harbour some resistant genes which could be used for crop improvement programmes.

* **Corresponding Author:** Shaheen Shah ✉ shaheenshah22@yahoo.com

Introduction

Tea (*Camellia sinensis*) is well-known for being the most refreshing health drink worldwide. Indian tea production started in 1983 in the state of Assam located in the North-Eastern part of India. Assam has enormous tea germplasm which serve as planting material for rest of the country (Balasaravanan *et al*., 2003).Sri Lanka and Kenya started their first tea plantations from the seeds of Assam, India. About 60% acreas of tea growing regions of the world had received its initial planting from Assam. Globally, each tea growing region has its own geographically specific clones (Mondal, 2014).In North-East India, tea clones are divided into standard (above average yield and quality), quality (high quality but average yield), and yield clones (average quality but high yield or above).Among TV (Tocklai variety) clones; TV1,TV23 and among garden series clones S3A3, Tinali are the four clones which are popular among the tea planters of North-East India for its quality and yield (Borbora *et al*., 1996). But due to repeated inbreeding of tea, there has been remarkable loss of desirable traits over the preceding years. Several research work were carried out to understand the genetic diversity of the tea germplasm using various genetic markers as detailed understanding help develop desirable traits for tea breeding (Kaundun *et al*., 2000). Molecular markers such as amplified fragment length polymorphism i.e, AFLP(Peng-Zhang Ji *et al*. ,2012),random amplification of polymorphic DNAs i.e, RAPD (Wachira *et al*., 1995; Kaundun *et al*.,2002; Francis *et al*. ,1997, Chen *et al*., 2002,Young Goo *et al*., 2002, Mewan *et al*. 2005),restriction fragment length polymorphisms (Matsumoto *et al*. ,1994) are used for evaluating the genetic variation in tea. Among these markers , RAPD molecular markers does not require specific knowledge of the DNA sequences of the test samples and therefore it is a simple, rapid and good method for differentiating germplasm on the basis of intra-specific (Corner *et al*. 2001) and inter-specific levels (Liang *et al*., 1994).

Helopeltis theivora inspite of being the most dreaded pest of tea in North East India causing devastation of variable degree to the tea clones from past to the recent times reducing tea production to 10-50% (Gurusubramanian *et al*., 2007), till date no work has been done from North-East India which explains the genetic variability in this plant in relation to *helopeltis* infestation though extensive work has been done on the tea germplasm. As knowledge on the readily available tea germplasm present in a particular area is vital for future crop improvement programme (Balasaravanan, 2003), so the present research study was carried out using RAPD markers to study the genetic variation among susceptible varieties of tea plants to *helopeltis* infestation in North-East India and hence to infer about the resistance of the plant to the notorious pest.

Materials and methods

Sample collection

Tea (*Camellia sinensis*) leaf samples of clones TV1, TV23, S3A3 and Tinali was collected from 7 leading tea gardens ; Ethelwood, Moran, Borborooah, Deohal, Tippuk, Lepetkotta and Anandabag tea estates located in Dibrugarh district, Assam, India. For the experimental study, a total of 27 genotypes which included 1 healthy plant (control) and 26 naturally infected samples were selected for the study.

DNA Extraction

Total genomic DNA of all the 27 genotypes of tea was isolated by the CTAB method with some modifications (Doyle and Doyle, 1990).

RAPD-PCR amplification

The isolated DNA was amplified using 10 decamer RAPD primers of OPA, OPB and OPF series (Table1) obtained from Operon Technologies (Inc. Alameda, California).The PCR reactions were performed in a final volume of 25 µl containing 10x Assay buffer (Fermentas),1.0 unit of Taq DNA polymerase (Fermentas),200µM each of dNTPs (Fermentas), 10 pmols/reaction of random primers, 50 ng of template DNA. This mixture was then placed in a Biometra T3 Thermocycler with initial cycle of denaturation (94°C,5 mins) followed by 42 cycles of denaturation(94°C,1 mins),annealing (37°C,1 mins)

and extention (72°C,2 mins) which is followed by a last cycle of extention of 7 mins. The amplified PCR products were then electrophoresed in 1.2% agarose (Himedia,molecular grade) gel which was later visualised by UV trans-illuminator (Alpha® Imager 2200, Alpha Innotech, USA) system and then photographed and documented.

Data analysis

To compare the efficiency of the RAPD markers in varietal identification, the discriminating power (D) of each primer was estimated based on Simpson's index of diversity (Hunter and Gaston 1988).Polymorphism information contents (PIC) for each RAPD primer was calculated using the formula:

$$PIC_i = 1 - \sum_{j=1}^{n} p_{ij}^2$$

where,

pij is the frequency of the jth allele for marker i summed across n patterns (Anderson *et al.*1993).

The presence of each band was scored as '1' and its absence as '0'. Faintly visible bands were not scored but a major band corresponding to faint band was considered for scoring. The scores (0 or 1) for each band obtained from photograph were entered in the form of a rectangular data matrix. The pair-wise association coefficients were calculated from qualitative data matrix using Jaccard's similarity coefficient for all 27 genotypes using computer program NTSYS (Numerical Taxonomy System) pc version 2.02e (Rohlf, 1998). The equation for calculating Jaccard's similarity coefficients 'f' between two samples A and B is :f = n_{xy} / (n_1 – n_z), where, n_{xy} = number of bands common to sample A and sample B,n_1 = total number of bands present in all samples, n_z = number of bands not present in sample A or B but found in other samples. Cluster analysis for the genetic distance was then carried out using UPGMA (Unweighted Pair Group Method with Arithmetic Mean) clustering method. The genetic distances obtained from cluster analysis through UPGMA were used to construct the dendrogram, depicting the relationships of the clones using the same NTSYS pc program.

Results

The 10 decamer random primers used to characterise 27 tea genotypes generated a total of 113 bands with 90 polymorphic and 23 monomorphic bands. The polymorphism percentage of the bands was found to be 78.82% with average banding pattern type of 16.9 indicating considerable variation among the clones. The highest polymorphism was found for primer D and H while lowest polymorphism was found for primer J. The discriminatory power 'D' ranged from 0.92 to 0.98 for all the 9 primers except primer J (0.81) indicating that the primers are efficient for identification between the varieties. The polymorphic information content (PIC) of 4 primers (B, D, E, H) ranged from 0.25 to 0.35 indicating a medium informative locus. The highest PIC was found for primer D while lowest was from primer J. The Jaccard's similarity coefficient calculated from RAPD data ranged from 0.32% for distant varieties (control and Tippuk Tinali) to 0.72% for closest varieties (Deohal S3A3 and Moran S3A3) with an average of 0.52% for single primer based RAPD patterns. Higher genetic similarity was found for within groups compared to between groups. Within groups, least similarity was observed for Tinali groups (41%) while S3A3 groups shows highest similarity of 72%.Between groups, minimum genetic similarity 37% were observed (i.e, maximum diversity of 63%) was observed among 3 groups namely, TV1 and TV23 groups (Moran TV1 and Deohal TV23), TV1 and S3A3 groups (Ethel. TV1 and Bor. S3A3) and TV1 and Tinali groups (Ethel. TV1 and Bor. Tinali) while maximum genetic similarity was observed between S3A3 and Tinali groups (67%, Ethel. S3A3 and Bor. Tinali).The dendogram constructed through NTSYS (pc 2.02) has generated 3 major clusters (cluster A,B and C). Cluster A consist of mainly Tinali groups (five samples) with two S3A3 plant samples. Cluster B consist of majority S3A3 samples (five) with TV23 (three) and TV1 (two) while cluster C consist of samples from all TV1 accession with one TV23 accession.

Discussion

From the RAPD study, the banding pattern generated

and the polymorphism reflected in these patterns was used to identify the 27 accessions consisting of 4 varieties (TV1, TV23, S3A3, tinali) collected from 7 tea estates of Dibrugarh district, Assam, India. All the 10 amplifying arbitrary RAPD primers produced polymorphic bands. Though, none of the single primers produced unique patterns for all the twenty seven genotypes, they could distinguish all the genotypes collectively. A combination of more than two primers can be selected to distinguish all the accessions. The ability of a primer to distinguish between unrelated strains can be determined by the number of types (pattern types) defined by the primer and the relative frequencies of their types. A single numerical index of discrimination 'D' (Hunter *et al.*, 1988) was calculated based on the probability that two unrelated genotypes amplified from the test population will be placed into different typing groups. The D value greater than 0.90 is desirable to distinguish between two unrelated strains(Looveran *et al.,* 1999).The value of D in this study ranged from 0.92 to 0.97 (primer A, B, C, D, E, F, G, H, I) for single primer based RAPD patterns except for 0.81 (primer J) (Table1). Polymorphic information content (PIC) of a primer determines the value of a marker by analyzing its linkage with other loci. PIC values exceeding 0.50 indicates locus to be highly informative, values between 0.50 to 0.25 indicates a medium informative locus while values below 0.25 indicates locus to be non- informative (Botstain *et al.* 1980).In the present study, PIC (Polymorphic information content) of 4 primers (B, D, E, H) (fig 1, 2, 3, 4) ranges from 0.25 (primer J) to 0.35 (primer C) indicating a medium informative locus. (Table1).

Table 1. Table showing Primer Sequence, Total number of allele, Polymorphic allele, Monomorphic allele, Polymorphism percentage, Banding Pattern, Primer Discriminatory power and Polymorphic Information Content (PIC) and respective average.

Primer	Sequence (5`→3`)	GC Content (%)	Annealing Temp	Total No.of Bands	No. of Polymor-morphic Bands	Number of Monomor-phic Bands	Polymor-phisms (%)	Banding pattern Type	Discrimin-atory Power D	PIC Values
Primer A	TGCCGAGCTG	70	37°C	12	10	2	83.33	19	0.97	0.20
Primer B	AATCGGGCTG	60	34°C	14	12	2	85.71	23	0.98	0.29
Primer C	AGCCAGCGAA	60	34°C	14	12	2	85.71	16	0.93	0.23
Primer D	TGGGGGACTC	70	37°C	9	9	0	100	15	0.96	0.35
Primer E	TCCGCTCTGG	70	37°C	9	6	3	66.66	19	0.97	0.25
Primer F	AGGGAACGAG	60	34°C	12	8	4	66.66	18	0.97	0.18
Primer G	ACCCCCGAAG	70	37°C	7	5	2	71.42	12	0.96	0.21
Primer H	GTGAGGCGTC	70	37°C	13	13	0	100	23	0.97	0.28
Primer I	CCGCATCTAC	60	34°C	12	10	2	83.33	13	0.92	0.23
Primer J	ACGACCGACA	60	34°C	11	5	6	45.45	11	0.81	0.15
Total	10			113	90	23		169		
				Avg. 11.3	Avg. 9.0	Avg. 2.3	Avg. 78.82	Avg. 16.9	Avg. 0.944	Avg. 0.23

Genetic similarity estimates based on RAPD banding patterns were calculated using method of Jaccard's coefficient analysis (Jaccard, 1908). The similarity coefficient matrix generated was subjected to algorithm "Unweighted Pair Group Method for Arithmetic Average (UPGMA)" to generate clusters using NTSYS 2.02 pc program (Rohlf, 1998).The Jaccard's pairwise similarity coefficient values ranged from 0.32% (control and Tip. Tinali(I)) to 0.72% (Deo.S3A3(I) and Mor.S3A3 (I)) with an average of 0.52**,** for single primer based RAPD patterns (Fig 5).The clusters constructed through NTSYS (2.02 pc) presented in the form of dendrogram has generated three major cluster (cluster A, B and C). Cluster A consists of 7 tea plant samples , two from S3A3 Groups, (Lep.S3A3, Ana.S3A3) and five from Tinali, (Ethel.Tinali(I) , Mor. Tinali(I) , Bor. Tinali(I) , Lep. Tinali(I) , and Ana. Tinali(I)) . Cluster B consist of 10 plant samples, two from TV1 (Lep. TV1(I) and Ana.TV1(I)) three from TV23((Bor.TV23(I).Ethel.TV23(I), and Tip.TV23(I)) and five from S3A3 (Mor.S3A3(I), Bor.S3A3(I), Deo.S3A3(I),

Tip.S3A3(I), and Lep.S3A3(I). Cluster C consist of 5 plant samples, four from TV1 (I) (Ethel., Deo., Mor., Tip.) and one from Deo.TV23 (I) (Fig 6).Five samples were found with exclusive lookout in the dendrogram that was Mor.TV23 that were recorded most distinguishing plant sample, while rest four sample found in two set with two sample in each. Two plant samples of the Tinali group separated at 48% similarity coefficient consisting of Deo.Tinali and Tip.Tinali. The effect on genetic similarity was also evident from comparatively higher similarity coefficient for within groups compared to between groups. Within groups least similarity was observed for Tinali groups 41 per cent ((I) Ethel. Tinali and Deo.Tinali and (II) Ethel .Tinali and Tip.Tinali), where as S3A3 groups' collection shows highest similarity (72%) between Mor.S3A3 and Bor. S3A3.High similarity value suggests that these tea clones have narrow gene pool within the genus Camellia (Mishra *et al.*, 2009).Between groups minimum genetic similarity 37 per cent were observed among the three groups i.e. TV1 and TV23 groups(between sample Mor.TV1 and Deo.TV23), TV1 and S3A3 (between sample Ethel.TV1 and Bor.S3A3) and TV1 and Tinali groups (Ethel.TV1 and Bor. Tinali) and maximum genetic similarity was observed between S3A3 and Tinali groups accession Ethel. S3A3 and Bor. Tinali (67%) followed by TV23 and S3A3 accession Ethel.TV23 and Ethel. S3A3.On the basis of Jaccard's similarity coefficient, on an average the relationships were TV1 Group were 50 per cent divers, TV23 group 58.4 per cent diverse, S3A3 group 56.29 per cent divers, and Tinali groups were 58.62 per cent diverse. In the present study, TV1 group shows minimum diversity which might be due to intensive selection program on this variety (Sui *et al.,* 2008) so are more prone to *helopeltis* infection as plant population which are genetically less diverse are more susceptible to pathogens (Bandyopadhyay, 2011).While Tinali group shows maximum diversity so are less prone to infestation as increase in host genetic diversity reduces the risk of infestation (Lively, 2010). Also from the cluster visualization in two dimensional (dendogram) and three dimensional view (principal component analysis i.e, PCA; fig 7)it is quite evident that different varieties (Tinali, S3A3, TV1) are different from each other, as they are occupying different clusters and hence must have some diversity in genes. But TV23 shared both clusters B and C which indicates TV23 to have some similarity with TV1 and S3A3. This might explain the higher susceptibility of TV1, TV23 and some S3A3 accession to *helopeltis* infestation. Again S3A3 besides sharing majority of cluster B is also sharing cluster A. This again suggest S3A3 to have some similar genes with Tinali. Thus, Tinali and some S3A3 samples are less prone to infestation and thus might harbour some resistant genes.

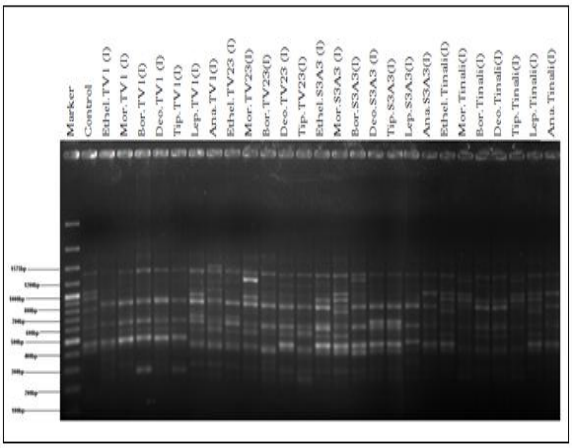

Fig. 1. RAPD profile using primer B from 27 accession of tea plants (varieties: TV1, TV23, S3A3 , Tinali) collected from 7 tea gardens (Ethelwood, Borborooah, Moran, Tippuk, Deohal, Anandabag, Lepetkotta) located in Dibrugarh district, Assam, India.

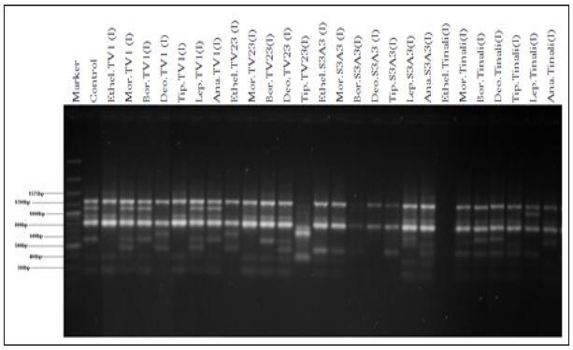

Fig. 2. RAPD profile using primer D from 27 accession of tea plants (varieties: TV1, TV23, S3A3 , Tinali) collected from 7 tea gardens (Ethelwood, Borborooah, Moran, Tippuk, Deohal, Anandabag, Lepetkotta) located in Dibrugarh district, Assam, India.

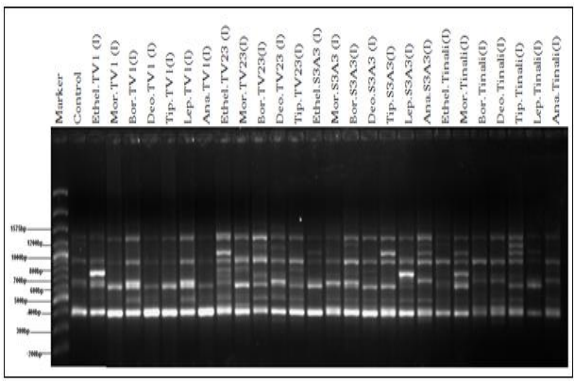

Fig. 3. RAPD profile using primer E from 27 accession of tea plants (varieties: TV1, TV23, S3A3 , Tinali) collected from 7 tea gardens (Ethelwood, Borborooah, Moran, Tippuk, Deohal, Anandabag, Lepetkotta) located in Dibrugarh district, Assam, India.

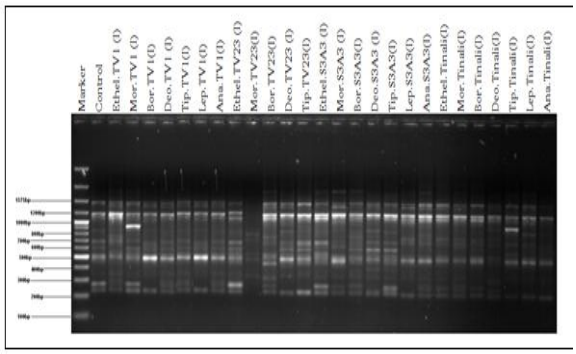

Fig. 4. RAPD profile using primer H from 27 accession of tea plants (varieties: TV1, TV23, S3A3 , Tinali) collected from 7 tea gardens (Ethelwood, Borborooah, Moran, Tippuk, Deohal, Anandabag, Lepetkotta) located in Dibrugarh district, Assam, India.

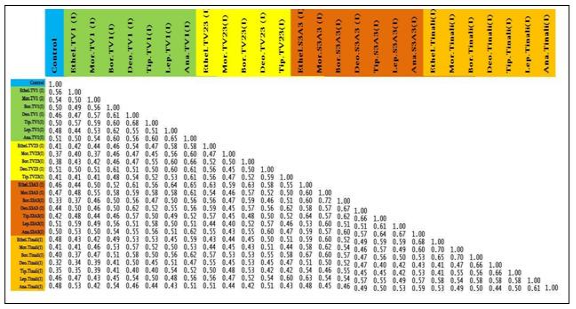

Fig. 5. Jaccard`s Similarity Coefficient generated using 10 RAPD primers profile for 27 accession of tea plants (varieties: TV1, TV23, S3A3, Tinali) collected from 7 gardens (Ethelwood, Borborooah, Moran, Tippuk, Deohal, Anandabag, Lepetkotta) located in Dibrugarh district , Assam, India.

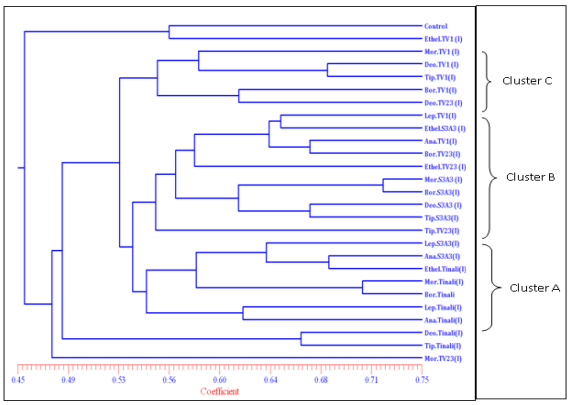

Fig. 6. Dendrogram generated using 10 RAPD primers profile for 27 accession of tea plants (varieties: TV1, TV23, S3A3, Tinali) collected from 7 gardens (Ethelwood, Borborooah, Moran, Tippuk, Deohal, Anandabag, Lepetkotta) located in Dibrugarh district, Assam, India.

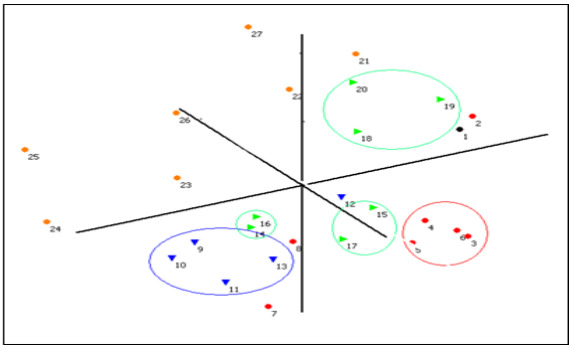

Fig. 7. PCA generated using 10 RAPD primers profile for 27 accession of tea plants (varieties: TV1, TV23, S3A3, Tinali) collected from 7 gardens (Ethelwood, Borborooah, Moran, Tippuk, Deohal, Anandabag, Lepetkotta) located in Dibrugarh district, Assam, India (numbers indicate the serial names of genotypes as in fig. 1).

Thus from the present study, RAPD markers has proved efficient tool in differentiating all 27 accessions consisting of 4 tea varieties (TV1, TV23, S3A3, Tinali) showing high polymorphism among them which determine their genetic diversity. Also the dendogram has placed the moderately susceptible varieties in separate cluster indicating that they might harbour some resistant genes. Future research in this direction may help in the development of marker assisted selection by creating sequence characterised amplified region(SCARs) from the RAPD markers thus improving the tea variety through creation of

helopeltis resistant clones which will ultimately help in future breeding and crop improvement programmes.

Acknowledgement

The authors acknowledge Director, Centre for Studies in Biotechnology, Dibrugarh University for providing all the facilities to carry out the study and DBT-NE project, Govt. of India for funding.

References

Anderson JA, Churchill GA, Autrique JE, Tanksley SD, Sorrells. 1993. Optimizing parental selection for genetic linkage maps. Genome **36,** 181-186.

Balasaravanan T, Pius PK, Kumar R, Raj Muraleedharan N, Shasany AK. 2003. Genetic diversity among South Indian tea germplasm using AFLP markers. Plant Sci **165,** 365-372. http://dx.doi.org/10.1016/S0168-9452(03)00196-1

Bandyopadhyay T. 2011. Molecular marker technology in Genetic improvement of tea. Inter Journ of Pla Breedi and Geneti **5(1),** 23-33. http://dx.doi.org/10.3923/ijpbg.2011.23.33

Borbora BC, Barua DN, Bera B. 1996 .Tea breeding at Tocklai. Two and a Bud **43(1),** 3-9.

Botstain D, White RL, Skolnick M, Dawid RW. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet **32,** 314-331.

Chen L, 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. J Hortic Sci Biotechnol **77,** 729-732.

Corner PJ, Wood BW. 2001.Identification of pecan cultivars and their genetic relatedness as determined by randomly amplified polymorphic DNA analysis. J Am Soc Hort Sci **126,** 474-480.

Doyle JJ, Doyle JL. 1990. A rapid total DNA preparation procedure from fresh plant tissue. Focus **12,** 13-15.

Francis NW, Wayne P, Robbie W. 1997. An assessment of genetic diversity among *Camellia sinensis* L. (cultivated tea) and its wild relatives based on RAPD and organelle specific STS. Heredity **78,** 603-611.

Gurusubramanian G, Bora S. 2007. Relative toxicity of some commonly used insecticides against adults of Helopeltis theivora Waterhouse (Miridae: Hemiptera) collected from Jorhat area tea plantations, South Assam, India. Resist. Pest Manage Newslett **17,** 8-12.

Hunter PR, Gaston MA. 1988. Numerical index of the discriminatory ability of typing system: an applied form of Simpson's index of diversity. JCM **26(11),** 2465-2466.

Jaccard A. 1908. Nouvelies researchers Socete Vaudoise de Sciences Naturalles **44,** 223-270.

Kaundun SS, Zhyvoloup A, Park YG. 2000. Evaluation of the genetic diversity among elite tea using RAPD markers. Euphyt **115 (1),** 7-16.

Kaundun SS, Park YG. 2002. Genetic structure of six Korean tea populations as revealed by RAPD-PCR markers. Crop Sci **42,** 594-601. http://dx.doi.org/10.2135/cropsci2002.5940

Liang GL, Zhou CQ, Lin MJ, Chen JY, Liu JS. 1994. Karyotype variation and evolution of sect Thea in Guizhou. Phytotaxonomic Sinica **32,** 308-315.

Lively CM. 2010. The effect of host genetic diversity on disease spread. Am Nat **175,** 149-152.

Looveran Van M, Ison CA, Leven M, Vandamme P, Martin IM, Vermeulen K, Renton A, Goossens H. 1999 .Evaluation of the discriminatory power of typing methods for Neisseria

gonorrhoeae. J Clin Microbiol **37(7),** 2183-2188.

Matsumoto S, Takeuchi A, Hayastsu M, Kondo S. 1994.Molecular cloning of phenylalanine ammonia-lyase cDNA and classification of varieties and cultivars of tea plants using the tea PAL cDNA probes. Theor Appl Genet **89,** 671-675. http://dx.doi.org/10.1007/BF00223703

Mewan KM, Liyanage AC, Everard JM, Gunasekare MTK, Karunanayaka. 2005. Studying genetic relationship among tea accessions in Sri Lanka using RAPD. Sri Lanka J Tea Sc **70,** 42-53.

Mishra RK, Chaudhury S, Ahmad A, Pradhan M, Siddiqi OT. 2009. Molecular analysis of tea clones (Camellia sinensis) using AFLP markers. Inter Journ of Integrat Biol **5(2),** 130-135.

Mondal TK. 2014. Breeding and biotechnology of tea and its wild species. Geneti and breedi **16,** 167-194.

Peng ZJ, Jun G, Ming ZL, Xing-QH. 2012. AFLP analysis of genetic variation among cloned, seed produced and wild Camellia sinensis in Yunnan, China. Pak J Bot 44**(6),** 1989-1992.

Rohlf JF. 1998. NTSYS-pc. Numerical Taxonomy and Multivariate Analysis System. Version 1.8. Exeter Software. New York.

Sui NI, Minghe YAO, Liang CHEN, Liping ZHAO, Xinchao W. 2008. Germplasm and breeding research of tea plant based on DNA marker approaches. Fronti of Agricult in China **2(2),** 200-207. http://dx.doi.org/10.1007/s11703-008-0043-1.

Wachira FN, Waugh R, Hackett CA , Powell W. 1995. Detection of genetic diversity in tea using RAPD markers. Genome **38,** 201-210.

Young Goo P, Kaundan, Zhyvoloup. 2002. Use of bulked genomic DNA-based RAPD methodology to assess the genetic diversity among abandoned Korean tea plantations. Gen Resourc Crop Evol **49,** 159-165. http://dx.doi.org/10.1023/A:1014706528972