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Assessment of Genetic Diversity in Tea Genotypes Through RAPD Primers

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Abstract: This study communicates the result of 24 promising tea genotypes evaluated through 11 Randomly Amplified Polymorphic DNA primers. High level of genetic diversity was observed on DNA level among the genotypes. The results of cluster analysis based on RAPDs revealed that four major groups could be recognized in tea grown in Mansehra District.

Key words: Tea, *Camellia sinensis*, genetic diversity, RAPD-DNA, cluster analysis

INTRODUCTION

Tea [*Camellia sinensis* (L.) Kuntze; (Usher, 1984)] is the most important non-alcoholic caffeine containing beverage of the world. It is an out-crossing species with woody perennial bushy species propagated both through shoot cutting and seed. Young leaves of the bush are plucked and processed in to Black and Green Tea. In Pakistan tea is emerging as a very promising crop because significant amount of foreign exchange is annually spent on its import. During the year 2004-2005, only, 134 thousand tones of tea of worth of Rs. 13202 million was imported to Pakistan (Anonymous, 2005). In recent past, efforts have been made to maximize tea cultivation in northern Pakistan especially in the Districts of Mansehra, Swat and Azad Kashmir. Selection of better genotypes, its characterization and production of improved clones for commercial cultivation is still needed for maximization of tea in Pakistan.

Like any other crop, breeding of improved tea genotypes needs the availability of genetically diverse germplasm and its proper evaluation in suitable agroecosystem. Like other crop, in tea also, morphological features were previously used to estimate genetic diversity but these traits were either limited in scope and mostly time consuming for evaluation. The recent developments in molecular biology especially the availability of unlimited number of molecular markers has provided the handy opportunities of utilizing Marker Assisted Selection for crop improvement (Paterson *et al.*, 1991). Due to the large number, easy handling and reliable scoring methods, the DNA based markers are considered as the best tools for determining phylogenetic relationships in plants.

Variety of molecular markers including Restriction Fragment Length Polymorphism (RFLP), Simple Sequence Repeat Primers (SSR) and Amplified Fragment Length Polymorphism (AFLP) have been used to study the extent of genetic variation in various crops (Karp, 1997; Vos *et al.*, 1995). Randomly Amplified Polymorphic DNA (RAPD) are relatively recent techniques, successfully used for elaboration of phylogenetic relationship crops of agronomic value (Dos Santos *et al.*, 1994; Thormann *et al.*, 1994; Link *et al.*, 1995). It was therefore decided to characterize tea genotypes a recently introduced crop in Pakistan, with the help of RAPD technology. This communication reports some of the results of the use of RAPD-primers for elaboration of the phylogenetic relationship of the tea genotypes grown in Mansehra District, Pakistan.

MATERIALS AND METHODS

Material used during the present study included 24 tea genotypes of different origins. Plant material were obtained from National Tea Research Institute (NTRI), Shinkiari, Mansehra and Unilever (Pvt.) Ltd. Icherian, Mansehra. Information regarding the genotypes used is given in Table 1. Experimentation was done both at the Institute of Biotechnology and Genetic Engineering, NWFP Agricultural University, Peshawar and Department of Genetics, Hazara University, Mansehra.

Leaf samples were used as a source for isolation of total genomic DNA and modified protocol of Weining and Langridge (1991) were used for DNA isolation. For removing RNA, DNA was treated with 40 µg RNase-A at 37°C for 1 h and the samples were stored at 4°C. For the use of PCR machine 1: 4 dilution of DNA was made

Table 1: Basic information regarding the tea genotypes used for estimation of genetic diversity at DNA level

Clone ID	Source of collection
J-1	National Tea Research Institute, Shinkari
J-2	National Tea Research Institute, Shinkari
SL-1	National Tea Research Institute, Shinkari
IN-1	National Tea Research Institute, Shinkari
T-1	National Tea Research Institute, Shinkari
T-2	National Tea Research Institute, Shinkari
P-3-1	Unilever (Pvt.) Ltd. Icherian
P-3-2	Unilever (Pvt.) Ltd. Icherian
P-5-1	Unilever (Pvt.) Ltd. Icherian
P-5-2	Unilever (Pvt.) Ltd. Icherian
P-8-1	Unilever (Pvt.) Ltd. Icherian
P-8-2	Unilever (Pvt.) Ltd. Icherian
P-9-1	Unilever (Pvt.) Ltd. Icherian
P-9-2	Unilever (Pvt.) Ltd. Icherian
Aa 29/88	National Tea Research Institute, Shinkari
Aa 26/80	National Tea Research Institute, Shinkari
Ab 7/66	National Tea Research Institute, Shinkari
Ab 24/80	National Tea Research Institute, Shinkari
Ac 34/21	National Tea Research Institute, Shinkari
Ac 29/5	National Tea Research Institute, Shinkari
Ad 18/9	National Tea Research Institute, Shinkari
Ad 3/4	National Tea Research Institute, Shinkari
Ba 44/28	National Tea Research Institute, Shinkari
Bf 2/6	National Tea Research Institute, Shinkari

in doubled distilled, deionized and autoclaved water. Eleven Randomly Amplified Polymorphic DNA primers (GL-A04, GL-C07, GL-D04, GL-D18, GL-E04, GL-E11, GL-F05, GL-G05, GL-G07, GL-H02 and GL-H09) obtained from GeneLink, Inc. NY 10532, USA) were used for DNA analyses. PCR reactions were carried out in 25 μ L reaction using standard protocols (Devos and Gale, 1992). Amplification conditions involved an initial denaturation step of 4 min at 94°C followed by 40 cycles each consisting of a denaturation step of 1 min at 94°C, followed by an annealing step of 1 min at 34°C and an extension step of 2 min at 72°C. The last cycle was followed by 7 min extension at 72°C. All amplification reactions were performed using the GeneAmp PCR system 2700 (Applied Biosystem). The amplification products were electrophoresed on 2.0% agarose/TBE gels and visualized by staining with ethidium bromide and viewed under UV light (Sambrook *et al.*, 1989).

For genetic diversity analysis, every scorable band was considered as single allele/locus and was scored 1 for presence or 0 for absence. The bivariate 1-0 data were used for estimation of genetic distances (G.D) following Unweighted Pair Group of Arithmetic Mean (UPGMA) procedures described by Nei and Li (1979). The dendrogram was prepared using computer program PopGene32 version 1.31 (<http://www.ualberta.ca/~fyeh/fyeh>).

RESULTS AND DISCUSSION

Result presented in this study is the first documented attempt for estimation of genetic diversity present in tea

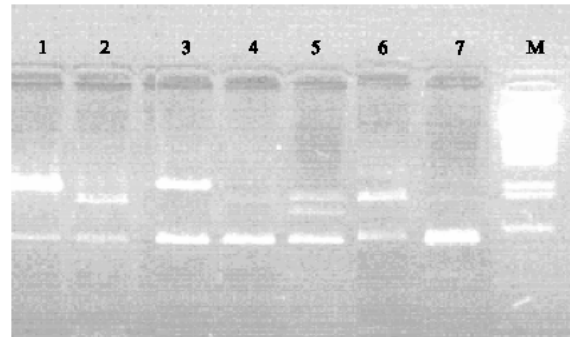


Fig. 1: Successful PCR amplification profile of seven tea genotypes using RAPD primer GL-C07 (M denotes molecular size marker (1 kb DNA ladder, GeneLink Inc. NY 10532, USA). Sizes of the fragments are indicated towards left from S. No. 1-7)

genotypes/clones grown in Pakistan. A successful PCR amplification profile of seven tea cultivars using RAPD primer GL-C07 is presented in Fig. 1. Molecular sizes of amplified fragments ranged between 250-1000 bp, approximately. In an earlier report Lai *et al.* (2001) observed approximately 200-1500 bp fragment size amplified using RAPD primers in different clones of tea, the finding are in conformity to our results. In the present study an average of 9.9 alleles per genotype were amplified using 11 RAPD primers. The results of genetic dissimilarity analyses (using UPGMA) showed that extensive genetic diversity (on average ranging from 0-100% among different genotypes) was present in all the 24 tea genotypes. The detection of high level genetic diversity observed in tea genotypes confirms the findings of Welsh and McClelland (1990) and Dos Santos *et al.* (1994) who demonstrated that RAPD markers are effective for visualizing high level of genetic polymorphism in plant species.

The Bivariate 1-0 data matrix generated dendrogram shows (Fig. 2) that the genotypes analyzed on DNA basis belong to four main clusters A, B, C and D, comprising 4, 8, 6 and 6 entries, respectively. Main cluster B was further subdivided into subgroups E and F, comprising 3 and 5 entries, respectively. On the basis of cluster analysis it was concluded that genotypes number 1 (J-1) and 6 (T-2) were genetically most diverse and hence recommended for the use of tea improvement program as the most promising genotypes.

Present results support and provide further strength the findings of Lai *et al.* (2001), Chen *et al.* (2000), Hu *et al.* (1995) and Hallden *et al.* (1994), who concluded that the DNA based markers especially RAPD markers could be effectively used for estimation of genetic distances among clones, genotypes, lines and

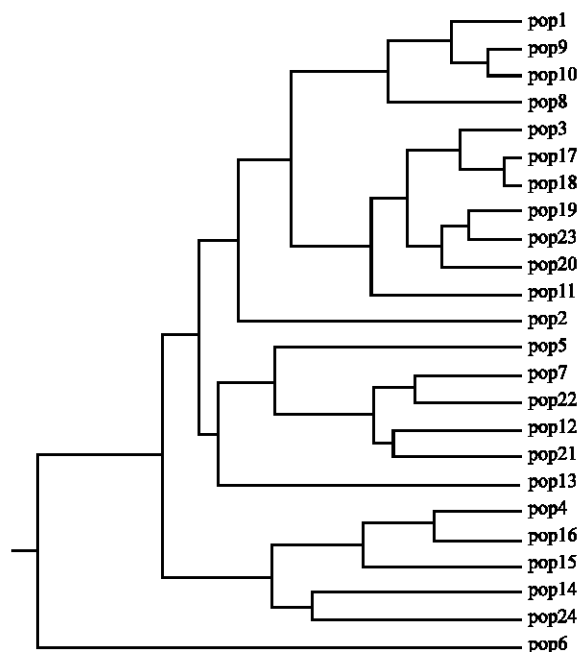


Fig. 2: DNA based phylogenetic relationship elaborated through the dendrogram of 24 tea clones, constructed through bivariate data matrix generated using 11 RAPD primers

hybrid combinations. Furthermore, the results concluded that more molecular data is required to elaborate the complete profile of the genetic diversity of the tea and select more promising lines for the improvement of tea crop in Pakistan.

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