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BRIEF COMMUNICATION

Detection of intra-clonal genetic variability in vegetatively propagated tea using RAPD markers

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Abstract

The role of random amplified polymorphic DNA (RAPD) markers in detecting intra-clonal genetic variability in vegetatively propagated UPASI-9 clone of tea (*Camellia sinensis*) was studied. Twenty five decamer primers were used, of which three did not amplify, three gave single bands and the rest of nineteen primers generated upto twelve bands (an average of 6.3 bands per primer). Twenty one primers exhibiting amplified products gave monomorphic banding patterns. Only one primer (OPE-17) gave a unique extra band of similar size in four plants.

Additional key words: *Camellia sinensis*, polymerase chain reaction (PCR).

Camellia sinensis (L.) O. Kuntze is propagated either by seed or stem cuttings. Due to cross pollination, heterogeneity in seed propagated plants is a limitation. Homogeneity in planting stocks is desirable requiring need for propagation through vegetative means. The objective in a vegetative propagation experiment is to obtain the genetically uniform population of a particular clone. Majority of the propagated plants from a desired clone are phenotypically similar, making it difficult to discriminate between the propagules which might be genetically different. It is of paramount importance, therefore, to assess the genetic constitution of the propagated plants. It will help to establish their genetic uniformity and stability before planning strategies for their further propagation and utilization, particularly in tea bushes, which are used for decades after planting. Although, somaclonal variations which come through tissue culture phase in plants have been reported much earlier (Larkin and Scrowcroft 1981), no systematic study related to the genetic variation of the plants propagated through nodal stem cuttings has been reported.

Two DNA based marker strategies namely restriction fragment length polymorphism (Atchinson *et al*. 1976) and random amplified polymorphic DNA (Williams *et al*. 1993) are commonly used in plants for genotype identification. Although hybridisation based technique is a choice in certain applications, it takes about 5 - 6 d to get the results. Furthermore, it is limited to detect only DNA sequences related to the probe and, therefore, is of limited use in detecting the random variations taking place during development. RAPD is devoid of such limitations and, therefore, is a major advance in plant identification. It has been applied to detect somaclonal variations (Godwin *et al*. 1997, Singh 1999, Rout and Das 2002) but it has rarely been applied to detect intra-clonal genetic variation on clonally propagated material. In this paper we report use of RAPD markers to discriminate apparantly highly homogenous vegetatively propagated plants of commercial tea clones.

Sixteen individual plants of UPASI-9, a tea clone representing an entire plot of 45 plants were used. These plants were propagated through nodal stem cuttings from a mother bush and were about seven years old. All plants look morphologically similar and are growing in the Institute's tea experimental farm (1 300 msl, 32°6' N, 76°18' E).

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Abbreviations: bp - base pair; RAPD - random amplified polymorphic DNA; RFLP - restriction fragment length polymorphism.

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Total genomic DNA of these plants was extracted from tender young leaves as described earlier (Saghai-Maroof *et al*. 1984). Fresh leaves (100 mg) were grinded in liquid nitrogen, 1 cm^3 of extraction buffer (2 % CTAB, 100 mM Tris-HCl, pH 8.0, 20 mM EDTA, 1.4 M NaCl, 1 % PVP) was added and then incubated at 65 ° C for 1 h. The equal volume of chloroform:isoamyl alcohol was added and the mixture centrifuged at 12 000 *g* for 20 min. DNA in the supernatant was precipitated by 0.6 volume of isopropanol. DNA was spooled out, dried briefly, dissolved in 0.2 cm^3 of 1X TE buffer and quantified spectrophotometrically (*Hitachi 150-20*, Tokyo, Japan). The decanucleotide primers used in this study were obtained from *Operon Technologies Inc*., Alameda, CA, USA. *Taq* DNA polymerase and its corresponding buffer (10 mM Tris-HCl, pH 9.0, 1.5 mM $MgCl_2$, 50 mM KCl, 0.01 % gelatine) was obtained from *Bangalore Genei Inc*., Bangalore, India. The dNTPs were from *United States Biochemicals*, Cleveland, OH, USA. The PCR reaction was carried out in a final volume of 0.025 cm^3 containing 100 μM each of four dNTPs, 7.5 pM primer, 20 ng of genomic DNA and 0.3 units of *Taq* DNA polymerase. Each reaction tube was loaded with equal volume of mineral oil. The amplification was performed in a *Robocycler* (*Stratagene*, La Jolla, CA, USA) programmed for one cycle of 4 min at 94 °C, 1 min at 37 °C and 2 min at 72 °C. The last extension cycle was programmed at 72 °C for 7 min. The amplified products were resolved in 1.4 % agarose gel using 1X TAE buffer. The gel was stained in ethidium bromide, visualised in a trans-illuminator and photographed.

In initial experiments a total of 25 primers were screened with a subset of tea samples. Out of 25 primers, three could not amplify the tea genomic DNA and two gave extremely faint and ambiguous bands. Twenty responding primers which produced clear cut and reproducible bands were further used to amplify genomic DNA from all sixteen plants. Three primers produced only single band, while rest of the seventeen primers produced 2 - 12 bands with an average of 6.3 bands per primer. The bands generated were primer dependant and were in the size range of 200 to 3 000 bp. An example of the RAPD patterns generated by representative primer sets OPA-5 and OPA-6 are shown in Fig. 1. All 20 responding primers, except one, revealed monomorphic banding patterns indicating a high degree of homogeneity in the tea population as expected. Thus the RAPD fingerprints appear to be stable in vegetatively propagated plants. Out of 25 primers screened, only one primer (OPE-17) revealed polymorphic bands. It was repeated at least three-times with the same results, indicating its reproducibility. An extra band of about 900 bp was present only in 4 plants numbering 6, 9, 15 and 16 (Fig. 2). The extra band in plant 6 is not clear in the photograph here but could be clearly seen in longer run of the agarose gel. There are many minor bands and some of them could be seen more intense in some lanes as compared to others (for example, lanes 5 and 11 in Fig. 2). It could be due to the differences in purity of the DNA templates of different genomic DNAs as has been reported earlier (Torres *et al*. 1993), although we gave similar treatments to all the DNAs.

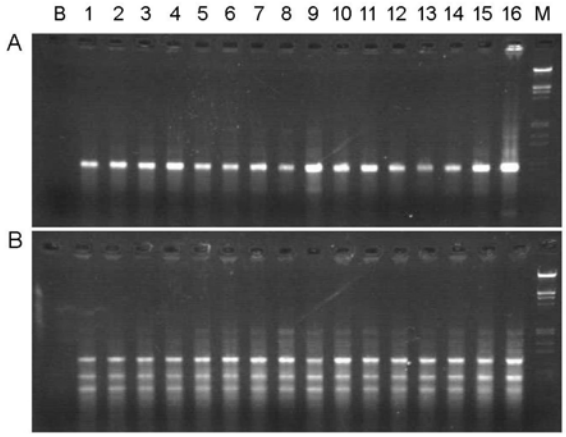

Fig. 1. RAPD profiles of 16 different plants of a tea clone generated with primers (*A* - OPA-5, 5'-AGGGGTCTTG-3'; *B* - OPA-6, 5'-GGTCCCTGAC-3'. M is molecular mass marker (λ *Hin*dIII-*Eco*R1). B is a control blank lane without template genomic DNA.

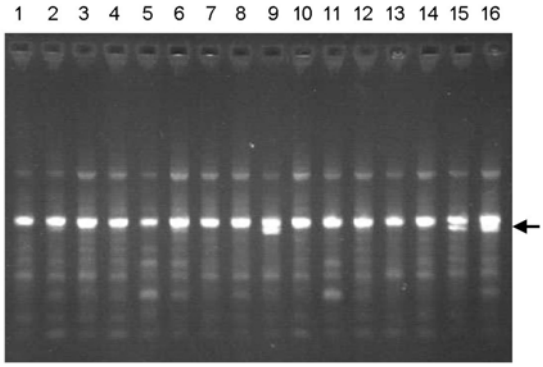

Fig. 2. Detection of polymorphic bands in tea as revealed by gel electrophoresis of RAPD products generated with primer OPE-17 (5'-CTACTGCCGT-3'). 1 - 16 are different plants of a tea clone. The appearance of novel band in lanes 6, 9, 15 and 16 is indicated by *arrow*.

A total of 120 bands were scored, of which only one extra band in four of the sixteen plants studied was observed and, therefore, the frequency of variation was found very low. It has been suggested (Williams 1993) that the altered RAPD patterns, *i.e*., the loss or gain of a band may be due to the variety of genomic alterations, such as a mutation in priming site or a deletion/insertion between the priming sites. Altered RAPD patterns could also be the result of altered competition for priming sites based on rearrangement/amplification in certain regions of genome (DeVerno *et al*. 1994). The factors leading to the observed

genetic variation and the frequency of their occurrence are not yet precisely known and need to be explored further. Santelices *et al*. (1995) have described causes and implications of intra-clonal variations in a study on red algal species and hypothesized that different species exhibit different amounts of intra-clonal genetic variation. The appearance of polymorphic loci in four plants in the present study could represent the pre-existing genetic variation in the mother bush. It is possible that all four plants which showed polymorphism could have come from the same branch of the mother bush. It should be noted that from one branch, five to six nodal cuttings are prepared which might have undergone mutational changes during early development of the branch. Intra-clonal variations in production of phytochemicals in leaves and stems, upon exposing the plants to ozone stress, have been reported earlier (Lavola *et al*. 1994). Somatic stability in all the vegetative parts, *i.e.*, flower, main stem, side stem, rossette leaves and stem leaves of *Achillea* have also been reported earlier using a synthetic RFLP probe (Wallner *et al*. 1996). Our results indicate that RAPD markers can be used to discriminate intra-clonal genetic variation in *Camellia sinensis* and probably in other plants propagated through nodal stem cuttings. To our knowledge this is the first report of a systematic evaluation of intra-clonal genetic variation in tea.

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