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Identification of molecular markers linked with differential flowering behaviour of mangoes in Andaman and Nicobar Islands

Genetic differences within and between geographic populations of an ecosystem are likely to be defined by the population-fluxing pattern as influenced by various ecological factors in the immediate past and historical pressure on the genome. The Andaman and Nicobar Islands in the Bay of Bengal (lat. 6°45′–13°41′N and long. 92°12′–93°51′E) comprise over 572 islands and rocks[1]. Due to the long history of cultivation in these islands, many cultivars of mango from both northern and southern India are known to exist. The varieties from northern India failed to flower in the absence of low temperature and low humidity. However, the varieties from southern India established due to their coincidence of the climatic requirement. Open pollination of these varieties resulted in many clones with differential flowering pattern due to the introgression of genes during hybridization. Most of these clones exhibited an erratic habit of bearing round the year. Flower bud differentiation takes place during May–June, August–September and November–December. Some clones exhibited multiple flowering while others though morphologically similar to the multiflowering clones, flowered only once[2].

In the existing context, a systematic and concerted effort to find the cause of differential flowering was found to be important. About 30 clones comprising 20 single-flowering and 10 multiple-flowering types were selected. Among them, three multiple-flowering local types (GL 1, GL 2 and GL 3), four parental single-flowering lines consisting of Neelam, Malgoa, Bangalora and Banganapalli and three local single-flowering types (HL 4, HL 6 and HL 12) were selected for intensive screening.

In the present study we report that the morphological character does not exhibit any variation between parental and open-pollinated clones. Qualitative and enzyme activities of these clones showed significant variation between them. This confirmed the influence of environment on flowering behaviour. However, the role of genic action on flowering cannot be ruled out. Thus to identify the influence of genes in flowering and molecular markers linked with the gene, variation at DNA level was analysed. Availability of reliable polymorphic markers often limits accurate estimation of genetic variation among individual populations[3]. Usual DNA-based techniques such as RFLP through Southern hybridization and use of micro-satellites are expensive; also use of latter required DNA sequence information[4]. PCR-based RAPD approach has been a handy and convenient alternative technique for investigation on genome mapping[5,6]. Genomic DNA was extracted from semi-mature leaves (1 g) by a modified CTAB method and purified by chloroform-isoamyl alcohol and RNAse treatment. In order to make a better representation of each clone, equal amount of DNA of 20 samples of each clone was pooled and the resulting bulked DNA samples were used for PCR–RAPD analysis. Quantified DNA was diluted to 20–40 ng/μl used for PCR–RAPD analysis. A set of 50 random decamer primers were selected from OPB, OPC, OPE, OPF, OPQ and OPX obtained from Bangalore Genei Pvt Ltd, Bangalore. Amplification was performed in 25 μl reaction mixture consisting of 40 ng genomic DNA, 10× reaction buffer with $MgCl_2$ 15 mM, 10 mM each of DATP, DCTP, DGTP and DTTP, 0.2 mM primer and 0.6U Taq polymerase (Bangalore Genei Pvt Ltd). PCR amplification was carried out on thermal cycler well blocks (MJ Research Inc., USA) in 0.2 ml micro-centrifuge tubes. The process was started with a 4 min initial denaturation at 94°C followed by 45 cycles of 1 min at 94°C for denaturation, 1 min at 37°C for annealing, 2 min at 72°C for extension and ended with a final 10 min extension at 72°C. Amplification products were maintained at 4°C until electrophoresis.

The reaction product was resolved by electrophoresis in a 1.5% agarose gel using 1× TBE buffer at 8 v/cm for 3 h. A 1 kb ladder (Bangalore Genei Pvt Ltd). All the 50 primers were tested at least twice for reproducibility of banding pattern and