Development, validation and demonstration of utility of EST-SSR markers

SSR markers being co-dominant and locus specific are the markers of choice in any molecular breeding programmes. In castor, no SSR markers have been reported. However, recently a good EST-Database has been available and this database is a useful source of SSR and could be used for developing genic SSR. Therefore, this activity was taken up to develop SSR markers as a prelude to use them in marker assisted selection.

A total of 64,338 EST sequences were analysed using sequencher software to eliminate the redundant ESTs. From the non-redundant EST dataset, 1959 ESTs containing Class I SSR markers belonging to dimeric (104), trimeric (100), tetrameric (9), pentameric (5) and hexameric (8) were identified. Primers for all the 226 EST-SSRs have been designed and 150 EST-SSRs have been synthesized. 80 of these EST-derived SSR primers were validated using the genomic DNA from two castor genotypes. Of these 62 primers gave the expected sized bands.

EST-SSR primers that gave expected sized amplicons in the preliminary step of verification of the primers were used for the genetic purity assessment of seven public sector safflower hybrids, DCH 32, DCH 177, DCH 519, GCH 2, GCH 4, GCH 5 and RHC 1. The strategy included identification of EST-SSR primer pairs that gave polymorphic bands using bulk DNA samples of the parents, parental polymorphism confirmation using individual plants, confirmation of the identified EST-SSRs primer pairs using bulk DNA of both hybrid and parents and validation of the identified EST-SSR marker using individual plant DNA samples. Details of the markers identified for each of the 7 hybrids is provided in Table 1. Fig 1 and 2 show the polymorphism pattern obtained with the individual plants of the parents of DCH32 (with CES22 primer) and DCH519 (with CES45) respectively. Fig 3 and 4 show the pattern for NH1 hybrid while Fig 5 and 6 show the pattern obtained with hybrid DSH129.

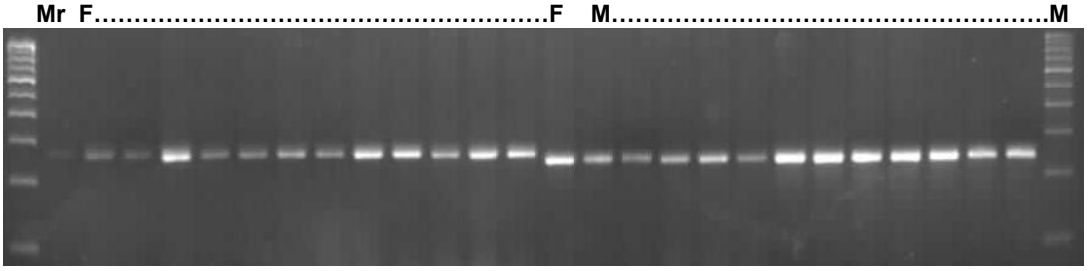

Figure 1. Female and Male individual DNA samples of DCH177 with primer CES22

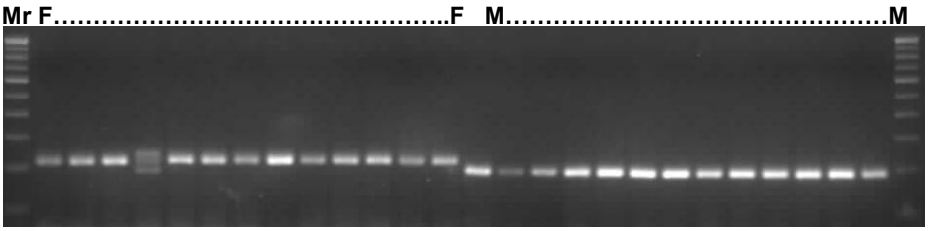

Figure 2. Female and Male individual DNA samples of DCH519 with primer CES45

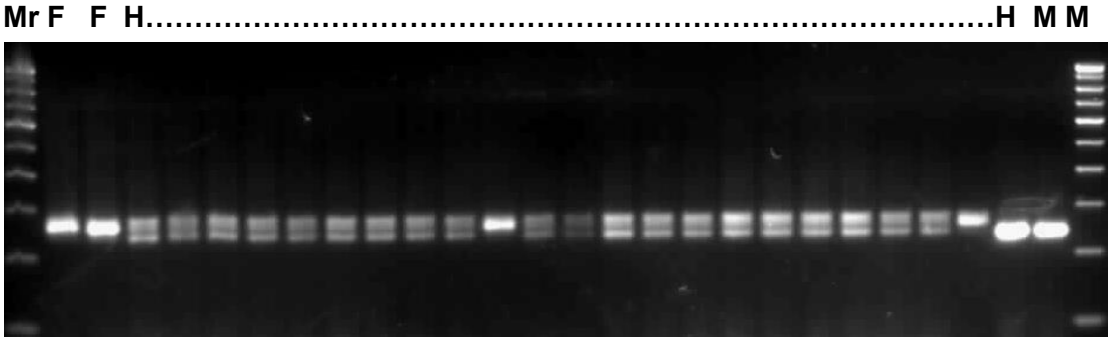

Figure 3. **Female, Hybrid and Male individual DNA samples of GCH4 with primer CES28**

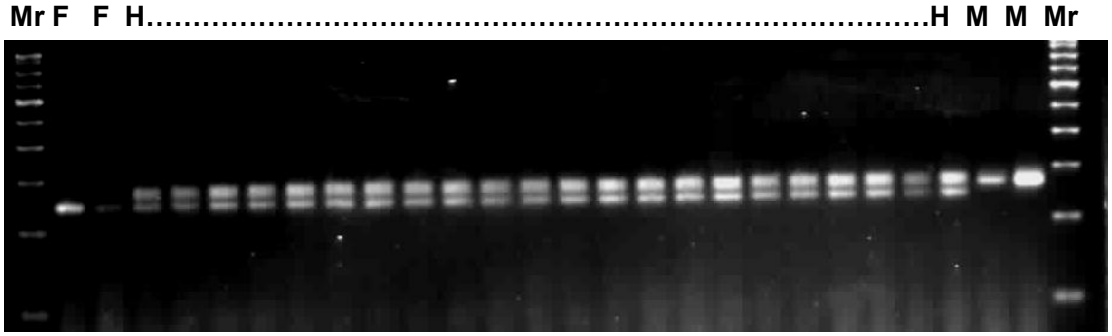

Figure 4. Female, Hybrid and Male individual DNA samples of GCH5 with primer CES22

Summary of the EST-SSR marker work:

- Primers for about 561 SSRs developed

- A total of 611 primer pairs were designed for the SSRs, having repeat length more than or equal to 20 nucleotides, of which a set of 130 markers were tested and 92 of these yielding robust amplicons were analyzed for their utility in genetic purity assessment of castor bean hybrids. Nine markers were able to detect polymorphism between the parental lines of nine commercial castor bean hybrids (DCH-32, DCH-177, DCH-519, GCH-2, GCH-4, GCH-5, GCH-6, GCH-7, and RHC-1), and their utility in genetic purity testing was demonstrated.

Table 1 Details of amplicons produced by EST-SSR primers identified for the genetic purity assessment of seven hybrids of castor

Hybrid	Primer	Size of female specific band (bp)	Size of male specific band (bp)
DCH 32	CES 28	260	250
DCH 177	CES 22, CES 55	270 , 210	260 , 200
DCH 519	CES 45	210	190
GCH 2	CES 9	200	210
GCH 4	CES 28, CES 45	270, 250	260 , 260
GCH 5	CES 22, CES 28	250, 250	260 , 260
RHC 1	CES 27, CES 28, CES 46	230, 250, 220	220, 240, 210