through enzyme purification was difficult and isoforms were likely to be missed. The cDNA screening and isolation were followed by analysis of all the encoded isoforms.

2 Materials and Methods

2.1 Polymerase chain reaction of pea isoamylase with degenerate primers

Degenerate primers were used to amplify genomic DNA extracted from embryos of pea plants (BC1/RR JI430 lines of John Innes Centre germplasm collection [6]). Primers sequences were 5'-G(^{G}/A/$_{T}$)A ATA C(^{A}/$_{C}$)T TCA A(^{C}/$_{T}$)T G(^{C}/$_{T}$)A ATC ATC C-3' and 5'-GG(^{A}/$_{G}$) TCA TT(^{A}/$_{G}$) CT(^{G}/A/$_{T}$) ATC ATG TCA AT-3'. The condition for polymerase chain reaction (PCR) was one cycle of template denaturation at 94°C for 5 min followed by denaturation at 94°C for 30 s, annealing at 50°C for 2 min and extension at 72°C for 3 min for 35 cycles. This was followed by a final extension at 72°C for 5 min. The resulting product of the PCR was purified with Qiagen gel extraction kit. It was then ligated into pGEM-T Easy vector (Promega) and inserted into *E. coli* DH5α. The PCR fragment was used as general isoamylase probe for screening of the cDNA library.

2.2 Polymerase chain reaction of potato Stisa2

This PCR was undertaken to amplify fragments from potato Stisa2 cDNA clone to be used as probes for pea cDNA library screening. The primers used were 5'-GAA TCC ATT AAC CAA TGA TTC GAA GGA AA-3' and 5'-CAA GAA TGA AAT AAA CTG GCC AAT CTG AGTC-3'. The programme used was one cycle of template denaturation at 94°C for 4 min followed by 35 cycles of denaturation at 94°C for 10 s, annealing of primers at 55°C for 20 s and elongation of primers at 72°C for 1 min. This was followed by a final cycle of elongation at 72°C for 3 min. The PCR product was run on agarose gel and purified with Qiagen gel extraction kit.

2.3 Probe for isoamylase isoform 3

A probe was prepared by cleaving the plasmid containing the cDNA of Stisa3 isoform of potato isoamylase [7] with *Ase*I and *Xho*I. All probes used for cDNA library screening were prepared using the random priming technique [8].

2.4 Screening of pea embryo cDNA library

Amplified BC1 pea embryo cDNA library [9], prepared in λZAP (Uni-ZAP XR, Stratagene) was plated out to the density of 1×10^6 plaque forming units (p.f.u.) per plate on lawns of *E. coli* XL1-Blue. Plaques were lifted on 120 mm nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany). The filters were soaked in denaturation solution, followed by prehybridisation and hybridisation with probes as described in *Sambrook et al.* [8] followed by washing at different temperatures as well as different washing buffer strengths. The filters were then exposed on X-ray film overnight at –70°C. Plaques from the positive spots were cored and kept in 500 μL SM buffer with few drops of chloroform. The positive plaques were re-plated and hybridisation was repeated twice more to confirm the positive clones. Phagemid of the positive clones were obtained from the library by *in vivo* excision using Rapid Excision Kit (Stratagene). The resulting plasmids containing the positive clones were extracted using Qiagen minipreps kit and followed by sequencing of the plasmids.

3 Results and Discussion

3.1 Screening of pea embryo cDNA library

A PCR fragment encoding isoamylase from pea, which was amplified using degenerate primers, was used for screening a cDNA library. The screening was done using amplified cDNA library of pea embryo, plated at density of 1×10^6 p.f.u. per plate. High stringency washes (0.1 × sodium chloride-citrate (SSC), 0.5%, w/v, sodium dodecyl sulphate (SDS), 65°C) were used. The screening resulted in isolation of a cDNA fragment with the size of 2.8 kb and encoded a polypeptide of 791 amino acids. A pairwise comparison using the BLAST 2 programme [10] with Su1 polypeptide of maize showed 82% similarity (72% identity), while comparison with Stisa1 of potato showed 85% similarity (76% identity). This clone was named *Psisa1*. It contained 129 nucleotides in the 5'-untranslated region and 299 nucleotides in the 3'-untranslated region. The 3'-untranslated region contained one putative polyadenylation signal (AATAAT) located at 52 bp upstream of the last polyadenylation site. The nucleotide preceding the initiation ATG codon conformed to the observation by *Joshi* [11] that has a purine base at the –3 position.

Screening the library using probes from regions of the cDNAs of *Stisa2* and *Stisa3* potato isoamylases was attempted using very low stringency washes (3 × SSC, 0.5%, w/v, SDS, 55°C). Screening with a probe from *Stisa2* gave ten positive plaques but only two were subcloned and sequenced after further screening. Both were eventually found to have the same nucleotide sequence and therefore concluded to be the same clone. Screening

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