Isolation and characterization of cDNAs encoding storage proteins of chickpea (Cicer arietinum L.)

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A cDNA library was constructed in λZAPII vector from poly(A)+ RNA isolated from developing seeds of chickpea (Cicer arietinum L.). Two cDNA clones encoding legumin protein were obtained by screening the library by plaque hybridization using a heterologous pea legumin cDNA probe. The pBluescript plasmids were excised from the phage clones and two clones designated pCSSK4 and pCSSK5 with inserts of 1.45 kb and 1.82 kb respectively were mapped by restriction and partially sequenced. The partial nucleotide sequence showed that these two cDNAs are not identical and showed sequence homology with the storage protein cDNA clones of other legumes.

Introduction

Chickpea is the second most important legume crop in the world. It is a major food legume in many developing countries of southeast Asia where it is an important source of protein for people and is likely to remain an important crop for undernourished people in poor environments. Hence, the technological thrust on chickpea, wherein research should focus on improvement in yield and seed quality. The nutritional quality of legume storage proteins is poor due to inherent deficiency of sulphur-containing amino acids (Boulter et al 1981). There have been many attempts to manipulate the balance of essential amino acids in important crops by traditional methods of plant breeding but improvement in nutritional quality have often come at the expense of yield and quality.

The storage proteins of various legumes have been characterized extensively in terms of their structure and genes including pea, soyabean, Vicia faba and Phaseolus vulgaris (Croy et al 1982; Diomoney and Casey 1987; De Pace et al 1991). The expression of these storage protein genes has also been studied in other plant systems including Petunia and tobacco (Sengupta-Gopalan et al 1985; Beachy et al 1985; Newbiggin et al 1990). However, there are no studies conducted on the isolation and characterization of storage proteins and their genes in chickpea. Studies on the structure and organization of storage protein genes and their cDNAs are thus essential for the genetic manipulation of these genes for balanced amino acid composition. Therefore, in the present study we have constructed a cDNA library and isolated cDNAs encoding storage proteins of chickpea.

2. Materials and methods

2.1 Construction of cDNA library

2.1a Isolation of poly(A)+ RNA: Total RNA was isolated from 5 g developing seeds of chickpea (18 DAF), after removing the testae aseptically by the acid guanidium thiocyanate phenol chloroform method (Chomczyski and Sachhi 1987). The quality and quantity of the total RNA was analysed by electrophoresis and spectrophotometric analysis. Poly(A)+ RNA was isolated from 2 mg total RNA using poly(U)+ discs according to the instructions of the manufacturer (Sigma). The poly(A)+ RNA was obtained directly in sterile water and its concentration determined (Sambrook et al 1989).

2.1b Northern blot: Approximately 2 µg poly(A)+ RNA was electrophoresed on 1% agarose formaldehyde gel,
transferred to a nylon membrane and hybridized with radiolabelled pea legumin cDNA probe, pDUB 8 (Lycett et al 1984a,b) according to standard protocols (Davis et al 1986; Sambrook et al 1989).

2.1c cDNA synthesis: The cDNA library was prepared from 5 μg poly(A)+ RNA using a cDNA synthesis kit (Stratagene). Following first and second strand synthesis (Gubler and Hoffman 1983), the cDNA ends were blunted using Klenow and size fractionated to obtain longer cDNAs using a Sephacryl S 300 column. The blunted cDNA ends were ligated with the phosphatased λZAP arms and packaged using Gigapack gold packaging extract (Stratagene). The packaged library was titered using Escherichia coli XL-1 Blue host cells, followed by amplification by pouring the library on 150 mm NZY plates. After appearance of plaques, the plates were overlaid with 10 ml SM buffer and kept at 4°C overnight with gentle shaking. The supernatant was decanted from the plates and the library was stored in 0.3% chloroform at 4°C in screw capped vials.

2.2 Screening cDNA library

The cDNA library was plated to obtain 10,000–20,000 plaques per 150 mm NZY plate. Initially 10 plates were poured and the plaques were lifted on to nylon membranes (Benton and Davis 1977) and hybridized with pea legumin probe as discussed previously. Screening was carried out at the primary, secondary and tertiary levels to identify and isolate the putative positive clones. From the positive clones the pBluescript plasmid was excised using the helper phage Ex assist and then transformed into E. coli SOLR cells (Short et al 1988) to obtain colonies on LB Amp plates.

2.3 Isolation of plasmid and Southern hybridization

Plasmid DNA was isolated from the transformants obtained on LB Amp plates (Stephen et al 1990). Insert size was determined by restriction with enzymes EcoRI and XhoI. The restricted and unrestricted plasmid DNA was electrophoresed, transferred to a nylon membrane (Southern 1975) and hybridized with legumin probe as discussed previously.