

**ISOLATION AND CHARACTERISATION OF A GENE ENCODING THE  
 $\Delta^1$ -PYRROLINE-5- CARBOXYLATE SYNTHETASE  
IN SUGARCANE (*SACCHARUM* spp HYBRID VAR. ROC22)**

By

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**KEYWORDS:** Sugarcane,  $\Delta^1$ -Pyrroline-5- Carboxylate Synthetase (P5CS) Gene, Proline, Water Stress.

**Abstract**

SUGARCANE variety ROC22 was used as the experimental material. Water stress treatment was performed on 4 to 5-leaf stage plants with a 25% polyethylene glycol (PEG) 6000 solution. A cDNA sequence for the ScP5CS sugarcane gene was isolated by homologous cloning. The sequence contained 2151 bp and an open reading frame of 716 amino acids (GenBank accession number EU005373). Comparing the sequence of ScP5CS with that of sugarcane P5CS reported in GenBank, the nucleotide sequence (EF155655) showed high identity (98%), but the deduced amino acid was only 92% identical. The deduced protein contained a putative ATP-binding site, putative leucine domains, a Glu-5-kinase domain, a putative NADPH-binding domain, a conserved GSA-DH domain and a feedback inhibition site. Besides, there were differences in the Glu-5-kinase domain from the reported deduced amino acid sequence of P5CS (ABM30223), but less for the P5CSs from rice (*Oryza sativa*) and wheat (*Triticum aestivum*). So we believe this gene to be a new gene of sugarcane P5CS.

**Introduction**

Proline is accumulated under drought and salinity stress in a number of plant species and it is thought to play an important role in plant cells for adaptation to water stress. Proline is synthesised via two routes from either glutamate (Glu) or ornithine (Orn) in eukaryotes, specifically in higher plants and its accumulation is mainly from Glu (Delauney and Verma, 1993).

High-level expression of  $\Delta^1$ -pyrroline-5-carboxylate synthetase (P5CS), a bifunctional enzyme that catalyses the first and second reactions of proline biosynthesis, which exhibit both  $\gamma$ -GK and GSA dehydrogenase activities, has been reported to increase salinity stress tolerance in transgenic tobacco plants (Kishor *et al.*, 1995).

In *Vigna aconitifolia* and *Arabidopsis*, the first two steps of proline biosynthesis from Glu are catalysed by P5CS (Hu *et al.*, 1992; Savouré *et al.*, 1995; Yoshida *et al.*, 1995).

Several genes encoding the enzymes in the route of proline biosynthesis have been identified in a number of plant species, namely *Arabidopsis thaliana*, petunias, *Triticum aestivum* L. and *Oryza sativa* L.. Functional analysis showed that all have been reported to be up regulated in response to water deprivation and/or salinity (Hare and Cress, 1997; Hare *et al.*, 1998; Yamada *et al.*, 2005; Ma, 2005; Igarashi *et al.*, 1997). The genes encoding P5CS have been cloned from

*Arabidopsis thaliana*, rice (*Oryza sativa*), wheat (*Triticum aestivum*), alfalfa (*Medicago sativa*), tomato (*Lycopersicon esculentum*), lettuce (*Lactuca sativa*), soybean (*Glycine max.*), radish (*Raphanus sativus*), grape (*Vitis vinifera*), and moth bean (*Vigna aconitifolia*).

In this paper, we report on the isolation and structure of the ScP5CS gene in sugarcane as well as the features of the encoded polypeptide.

## Materials and methods

### Plant growth and salt-stress treatment

Seed cane of sugarcane variety ROC22 was germinated and grown in the laboratory. Water stress treatment was performed when the plants had developed 4–5 leaves. The plants were treated with a 25% polyethylene glycol (PEG) 6000 solution. Control plants were treated with deionised water. Leaf samples were taken for RNA extraction 18–24 h after treatment.

### Cloning and sequencing of P5CS cDNA from sugarcane leaves

Total RNA was extracted from young leaves from both treated and control plants. PCR amplification was conducted using the procedures described by Wang and Fang (2002). The P5CS cDNA fragment was amplified from the total pool of cDNA, using gene specific primers designed with reference to the known P5CS gene sequences (GenBank accession number: EF155655, AY574031, D49714, AY888045, DQ864376).

The cDNA for PCR was synthesised in a standard first strand reaction using 0.5–5 µg of total RNA from leaves with 20 units of avian myeloblastosis virus reverse (AMV) transcriptase (Takara). The cDNA was subjected to 35 cycles of amplification in a 25 µL reaction mixture containing 0.4 µM of each primer, 0.2 mM each of dATP, dTTP, dGTP, and dCTP, 10×PCR Buffer (MgCl<sub>2</sub> plus) and 1.25 units of *Taq* DNA polymerase.

Each amplification cycle consisted of 3 min at 94°C, then 1 min of denaturation at 94°C, 40 s of annealing at 53°C, and 90 s of extension at 72°C, with a final extension for 10 min at 72°C. The PCR products (10 µL) were size fractionated by electrophoresis through 1% agarose gels and extracted from the gels. The amplified cDNA was cloned and sequenced, and named ScP5CS.

### Sequence analysis

The analyses of DNA and protein sequences were performed using DNAMAN Software package and BLAST program of the NCBI. Homology alignment was performed using the Clustalx 1.83 and ClustalW program.

## Results

### Isolation, sequencing, and characterisation of sugarcane ScP5CS cDNA

The complete nucleotide sequence of a P5CS cDNA clone, ScP5CS, was cloned. The sequence of 2151 bp contained a single major open reading frame encoding a polypeptide of 77762.0 Da for a putative protein of 716 amino acids with a calculated isoelectric point of 6.29 (GenBank accession number EU005373).

Translation would be initiated from the first ATG codon, at the 5' end of the coding strand. Comparing the sequence of ScP5CS with that of sugarcane P5CS reported in GenBank revealed sequence similarity of 98%, although the deduced amino acid was only 92% similar. However, the isolated cDNA showed 85% and 84% overall sequence similarity to those of rice (*Oryza sativa*) and wheat (*Triticum aestivum*) respectively.

Interestingly, the putative ATP-binding site, putative leucine domains, Glu-5-kinase domain, putative NADPH-binding domain, conserved GSA-DH domain and feedback inhibition site were present in each of the enzymatic domains of sugarcane ScP5CS (Chiang *et al.*, 1995; Ma, 2005; Hu *et al.*, 1999). There were more differences in Glu-5-kinase domain from the deduced amino acid of the reported sugarcane P5CS (ABM30223), but less for the P5CSs from rice (*Oryza sativa*) and wheat (*Triticum aestivum*) (Figure 1).

AAS89034	MA <span style="background-color: #e0f0ff;">SVDP</span> PSRSFVRDVKRVI <span style="background-color: #e0f0ff;">IKVGTAV</span> VS <span style="background-color: #e0f0ff;">RQD</span> GRLALGRV <span style="background-color: #e0f0ff;">GALCEQVKELNSL</span> G <span style="background-color: #e0f0ff;">YEV</span> ILVTS <span style="background-color: #e0f0ff;">GAVGVGRQRLRYRKL</span> VSS <span style="background-color: #e0f0ff;">F</span>	80
BAA19916	MA <span style="background-color: #e0f0ff;">SVDP</span> PSRSFVRDVKRVI <span style="background-color: #e0f0ff;">IKVGTAV</span> VS <span style="background-color: #e0f0ff;">RQD</span> GRLALGRV <span style="background-color: #e0f0ff;">GALCEQVKELNSL</span> G <span style="background-color: #e0f0ff;">YEV</span> ILVTS <span style="background-color: #e0f0ff;">GAVGVGRQRLRYRKL</span> VSS <span style="background-color: #e0f0ff;">F</span>	80
AA35536	MA <span style="background-color: #e0f0ff;">GAD</span> PNRSF <span style="background-color: #e0f0ff;">MKD</span> VKRI <span style="background-color: #e0f0ff;">IKVGTAV</span> IT <span style="background-color: #e0f0ff;">RND</span> GRLALGR <span style="background-color: #e0f0ff;">I</span> G <span style="background-color: #e0f0ff;">ALCEQVKDLNAQ</span> G <span style="background-color: #e0f0ff;">YEV</span> IMVTS <span style="background-color: #e0f0ff;">GAVGVGRQRLRYRKL</span> VSS <span style="background-color: #e0f0ff;">F</span>	80
BAD97364	MA <span style="background-color: #e0f0ff;">GAD</span> PNRSF <span style="background-color: #e0f0ff;">MKD</span> VKRI <span style="background-color: #e0f0ff;">IKVGTAV</span> IT <span style="background-color: #e0f0ff;">RND</span> GRLALGR <span style="background-color: #e0f0ff;">I</span> G <span style="background-color: #e0f0ff;">ALCEQVKDLNAQ</span> G <span style="background-color: #e0f0ff;">YEV</span> IMVTS <span style="background-color: #e0f0ff;">GAVGVGRQRLRYRKL</span> VSS <span style="background-color: #e0f0ff;">F</span>	80
ABM30223	MA <span style="background-color: #e0f0ff;">TVDR</span> TRTF <span style="background-color: #e0f0ff;">MKD</span> VKRV <span style="background-color: #e0f0ff;">IKVGTAV</span> V <span style="background-color: #e0f0ff;">TTHD</span> GRLALGR <span style="background-color: #e0f0ff;">I</span> G <span style="background-color: #e0f0ff;">ALCEQVKELNALRYE</span> VI <span style="background-color: #e0f0ff;">I</span> VTS <span style="background-color: #e0f0ff;">GAVGVGRQRLRYRKL</span> VSS <span style="background-color: #e0f0ff;">F</span>	80
ABS32296	MA <span style="background-color: #e0f0ff;">TVDR</span> TRTF <span style="background-color: #e0f0ff;">MKD</span> VKRV <span style="background-color: #e0f0ff;">IKVGTAV</span> V <span style="background-color: #e0f0ff;">TTHD</span> GRLALGR <span style="background-color: #e0f0ff;">I</span> G <span style="background-color: #e0f0ff;">ALCEQVKELNALRYE</span> VI <span style="background-color: #e0f0ff;">I</span> VTS <span style="background-color: #e0f0ff;">GAVGVGRQRLRYRKL</span> VSS <span style="background-color: #e0f0ff;">F</span>	80
Consensus	ma d r dvkr iikvgtav dgrlalgr galceqvkl n yevi vtsgavgvgr ql yrklv ssf	
AAS89034	<span style="background-color: #e0f0ff;">ADLQK</span> PQ <span style="background-color: #e0f0ff;">MEL</span> DG <span style="background-color: #e0f0ff;">KACA</span> AVG <span style="background-color: #e0f0ff;">QSG</span> LMALYDML <span style="background-color: #e0f0ff;">FN</span> QLDVSS <span style="background-color: #e0f0ff;">QLLV</span> TD <span style="background-color: #e0f0ff;">SDF</span> ENPK <span style="background-color: #e0f0ff;">FRE</span> QLTET <span style="background-color: #e0f0ff;">VES</span> LL <span style="background-color: #e0f0ff;">DL</span> KVIP <span style="background-color: #e0f0ff;">FN</span> END <span style="background-color: #e0f0ff;">AIS</span>	160
BAA19916	<span style="background-color: #e0f0ff;">ADLQK</span> PQ <span style="background-color: #e0f0ff;">MEL</span> DG <span style="background-color: #e0f0ff;">KACA</span> AVG <span style="background-color: #e0f0ff;">QSG</span> LMALYDML <span style="background-color: #e0f0ff;">FN</span> QLDVSS <span style="background-color: #e0f0ff;">QLLV</span> TD <span style="background-color: #e0f0ff;">SDF</span> ENPK <span style="background-color: #e0f0ff;">FRE</span> QLTET <span style="background-color: #e0f0ff;">VES</span> LL <span style="background-color: #e0f0ff;">DL</span> KVIP <span style="background-color: #e0f0ff;">FN</span> END <span style="background-color: #e0f0ff;">AIS</span>	160
AA35536	<span style="background-color: #e0f0ff;">ADLQK</span> PQ <span style="background-color: #e0f0ff;">MEL</span> DG <span style="background-color: #e0f0ff;">KACA</span> AVG <span style="background-color: #e0f0ff;">QSG</span> LMALYDML <span style="background-color: #e0f0ff;">FT</span> QLDVSS <span style="background-color: #e0f0ff;">QLLV</span> TD <span style="background-color: #e0f0ff;">SDF</span> NSN <span style="background-color: #e0f0ff;">FR</span> ER <span style="background-color: #e0f0ff;">L</span> RET <span style="background-color: #e0f0ff;">VES</span> LL <span style="background-color: #e0f0ff;">EL</span> RVIP <span style="background-color: #e0f0ff;">FN</span> END <span style="background-color: #e0f0ff;">AIS</span>	160
BAD97364	<span style="background-color: #e0f0ff;">ADLQK</span> PQ <span style="background-color: #e0f0ff;">MEL</span> DG <span style="background-color: #e0f0ff;">KACA</span> AVG <span style="background-color: #e0f0ff;">QSG</span> LMALYDML <span style="background-color: #e0f0ff;">FT</span> QLDVSS <span style="background-color: #e0f0ff;">QLLV</span> TD <span style="background-color: #e0f0ff;">SDF</span> NSN <span style="background-color: #e0f0ff;">FR</span> ER <span style="background-color: #e0f0ff;">L</span> RET <span style="background-color: #e0f0ff;">VES</span> LL <span style="background-color: #e0f0ff;">EL</span> RVIP <span style="background-color: #e0f0ff;">FN</span> END <span style="background-color: #e0f0ff;">AIS</span>	160
ABM30223	<span style="background-color: #e0f0ff;">ADLQK</span> PQ <span style="background-color: #e0f0ff;">MEL</span> DG <span style="background-color: #e0f0ff;">KACA</span> AVG <span style="background-color: #e0f0ff;">QSG</span> LMALYDML <span style="background-color: #e0f0ff;">XT</span> QLDVSS <span style="background-color: #e0f0ff;">QLLV</span> TD <span style="background-color: #e0f0ff;">NDF</span> ENPN <span style="background-color: #e0f0ff;">FR</span> ER <span style="background-color: #e0f0ff;">L</span> RET <span style="background-color: #e0f0ff;">VES</span> LL <span style="background-color: #e0f0ff;">DL</span> KVIP <span style="background-color: #e0f0ff;">FN</span> END <span style="background-color: #e0f0ff;">AIS</span>	160
ABS32296	<span style="background-color: #e0f0ff;">ADLQK</span> PQ <span style="background-color: #e0f0ff;">MEL</span> DG <span style="background-color: #e0f0ff;">KACA</span> AVG <span style="background-color: #e0f0ff;">QSG</span> LMALYDML <span style="background-color: #e0f0ff;">FT</span> QLDVSS <span style="background-color: #e0f0ff;">QLLV</span> TD <span style="background-color: #e0f0ff;">SDF</span> ENPN <span style="background-color: #e0f0ff;">FR</span> ER <span style="background-color: #e0f0ff;">L</span> RET <span style="background-color: #e0f0ff;">VES</span> LL <span style="background-color: #e0f0ff;">DL</span> KVIP <span style="background-color: #e0f0ff;">FN</span> END <span style="background-color: #e0f0ff;">AIS</span>	160
Consensus	adlqkqmeldgkacaavgqsglmalydml qldvsssllvtd df n fre l etvesll l v pifnendais	
AAS89034	<span style="background-color: #e0f0ff;">TRKAP</span> YED <span style="background-color: #e0f0ff;">SSG</span> I <span style="background-color: #e0f0ff;">S</span> W <span style="background-color: #e0f0ff;">D</span> NS <span style="background-color: #e0f0ff;">L</span> AG <span style="background-color: #e0f0ff;">L</span> LA <span style="background-color: #e0f0ff;">E</span> LK <span style="background-color: #e0f0ff;">AD</span> LL <span style="background-color: #e0f0ff;">I</span> LL <span style="background-color: #e0f0ff;">S</span> D <span style="background-color: #e0f0ff;">V</span> D <span style="background-color: #e0f0ff;">G</span> LY <span style="background-color: #e0f0ff;">G</span> PP <span style="background-color: #e0f0ff;">S</span> EP <span style="background-color: #e0f0ff;">S</span> SK <span style="background-color: #e0f0ff;">I</span> I <span style="background-color: #e0f0ff;">H</span> T <span style="background-color: #e0f0ff;">Y</span> I <span style="background-color: #e0f0ff;">K</span> E <span style="background-color: #e0f0ff;">K</span> H <span style="background-color: #e0f0ff;">Q</span> Q <span style="background-color: #e0f0ff;">E</span> IT <span style="background-color: #e0f0ff;">F</span> G <span style="background-color: #e0f0ff;">D</span> K <span style="background-color: #e0f0ff;">S</span> R <span style="background-color: #e0f0ff;">V</span> G <span style="background-color: #e0f0ff;">R</span> G <span style="background-color: #e0f0ff;">G</span>	238
BAA19916	<span style="background-color: #e0f0ff;">TRKAP</span> YED <span style="background-color: #e0f0ff;">SSG</span> I <span style="background-color: #e0f0ff;">S</span> W <span style="background-color: #e0f0ff;">D</span> NS <span style="background-color: #e0f0ff;">L</span> AG <span style="background-color: #e0f0ff;">L</span> LA <span style="background-color: #e0f0ff;">E</span> LK <span style="background-color: #e0f0ff;">AD</span> LL <span style="background-color: #e0f0ff;">I</span> LL <span style="background-color: #e0f0ff;">S</span> D <span style="background-color: #e0f0ff;">V</span> D <span style="background-color: #e0f0ff;">G</span> LY <span style="background-color: #e0f0ff;">G</span> PP <span style="background-color: #e0f0ff;">S</span> EP <span style="background-color: #e0f0ff;">S</span> SK <span style="background-color: #e0f0ff;">I</span> I <span style="background-color: #e0f0ff;">H</span> T <span style="background-color: #e0f0ff;">Y</span> I <span style="background-color: #e0f0ff;">K</span> E <span style="background-color: #e0f0ff;">K</span> H <span style="background-color: #e0f0ff;">Q</span> Q <span style="background-color: #e0f0ff;">E</span> IT <span style="background-color: #e0f0ff;">F</span> G <span style="background-color: #e0f0ff;">D</span> K <span style="background-color: #e0f0ff;">S</span> R <span style="background-color: #e0f0ff;">V</span> G <span style="background-color: #e0f0ff;">R</span> G <span style="background-color: #e0f0ff;">G</span>	238
AA35536	<span style="background-color: #e0f0ff;">TRKAP</span> YED <span style="background-color: #e0f0ff;">SSG</span> I <span style="background-color: #e0f0ff;">S</span> W <span style="background-color: #e0f0ff;">D</span> NS <span style="background-color: #e0f0ff;">L</span> AG <span style="background-color: #e0f0ff;">L</span> LA <span style="background-color: #e0f0ff;">E</span> LK <span style="background-color: #e0f0ff;">AD</span> LL <span style="background-color: #e0f0ff;">I</span> LL <span style="background-color: #e0f0ff;">S</span> D <span style="background-color: #e0f0ff;">V</span> D <span style="background-color: #e0f0ff;">G</span> LY <span style="background-color: #e0f0ff;">G</span> PP <span style="background-color: #e0f0ff;">S</span> EP <span style="background-color: #e0f0ff;">S</span> SK <span style="background-color: #e0f0ff;">L</span> I <span style="background-color: #e0f0ff;">H</span> T <span style="background-color: #e0f0ff;">Y</span> I <span style="background-color: #e0f0ff;">K</span> E <span style="background-color: #e0f0ff;">K</span> H <span style="background-color: #e0f0ff;">Y</span> H <span style="background-color: #e0f0ff;">E</span> IT <span style="background-color: #e0f0ff;">F</span> G <span style="background-color: #e0f0ff;">D</span> K <span style="background-color: #e0f0ff;">S</span> R <span style="background-color: #e0f0ff;">V</span> G <span style="background-color: #e0f0ff;">R</span> G <span style="background-color: #e0f0ff;">G</span>	238
BAD97364	<span style="background-color: #e0f0ff;">TRKAP</span> YED <span style="background-color: #e0f0ff;">SSG</span> I <span style="background-color: #e0f0ff;">S</span> W <span style="background-color: #e0f0ff;">D</span> NS <span style="background-color: #e0f0ff;">L</span> AG <span style="background-color: #e0f0ff;">L</span> LA <span style="background-color: #e0f0ff;">E</span> LK <span style="background-color: #e0f0ff;">AD</span> LL <span style="background-color: #e0f0ff;">I</span> LL <span style="background-color: #e0f0ff;">S</span> D <span style="background-color: #e0f0ff;">V</span> D <span style="background-color: #e0f0ff;">G</span> LY <span style="background-color: #e0f0ff;">G</span> PP <span style="background-color: #e0f0ff;">S</span> EP <span style="background-color: #e0f0ff;">S</span> SK <span style="background-color: #e0f0ff;">L</span> I <span style="background-color: #e0f0ff;">H</span> T <span style="background-color: #e0f0ff;">Y</span> I <span style="background-color: #e0f0ff;">K</span> E <span style="background-color: #e0f0ff;">K</span> H <span style="background-color: #e0f0ff;">Y</span> H <span style="background-color: #e0f0ff;">E</span> IT <span style="background-color: #e0f0ff;">F</span> G <span style="background-color: #e0f0ff;">D</span> K <span style="background-color: #e0f0ff;">S</span> R <span style="background-color: #e0f0ff;">V</span> G <span style="background-color: #e0f0ff;">R</span> G <span style="background-color: #e0f0ff;">G</span>	238
ABM30223	<span style="background-color: #e0f0ff;">TRKAP</span> YED <span style="background-color: #e0f0ff;">SSG</span> I <span style="background-color: #e0f0ff;">S</span> W <span style="background-color: #e0f0ff;">D</span> NS <span style="background-color: #e0f0ff;">L</span> AG <span style="background-color: #e0f0ff;">L</span> LA <span style="background-color: #e0f0ff;">E</span> LK <span style="background-color: #e0f0ff;">AD</span> LL <span style="background-color: #e0f0ff;">I</span> LL <span style="background-color: #e0f0ff;">S</span> D <span style="background-color: #e0f0ff;">V</span> D <span style="background-color: #e0f0ff;">G</span> LY <span style="background-color: #e0f0ff;">G</span> PP <span style="background-color: #e0f0ff;">S</span> EP <span style="background-color: #e0f0ff;">S</span> SK <span style="background-color: #e0f0ff;">I</span> I <span style="background-color: #e0f0ff;">H</span> T <span style="background-color: #e0f0ff;">Y</span> I <span style="background-color: #e0f0ff;">K</span> E <span style="background-color: #e0f0ff;">K</span> H <span style="background-color: #e0f0ff;">H</span> NG <span style="background-color: #e0f0ff;">D</span> H <span style="background-color: #e0f0ff;">F</span> G <span style="background-color: #e0f0ff;">G</span> E <span style="background-color: #e0f0ff;">V</span> H <span style="background-color: #e0f0ff;">V</span> L <span style="background-color: #e0f0ff;">V</span> E <span style="background-color: #e0f0ff;">E</span>	240
ABS32296	<span style="background-color: #e0f0ff;">TRKAP</span> YED <span style="background-color: #e0f0ff;">SSG</span> I <span style="background-color: #e0f0ff;">S</span> W <span style="background-color: #e0f0ff;">D</span> NS <span style="background-color: #e0f0ff;">L</span> AG <span style="background-color: #e0f0ff;">L</span> LA <span style="background-color: #e0f0ff;">E</span> LK <span style="background-color: #e0f0ff;">AD</span> LL <span style="background-color: #e0f0ff;">I</span> LL <span style="background-color: #e0f0ff;">S</span> D <span style="background-color: #e0f0ff;">V</span> D <span style="background-color: #e0f0ff;">G</span> LY <span style="background-color: #e0f0ff;">G</span> PP <span style="background-color: #e0f0ff;">S</span> EP <span style="background-color: #e0f0ff;">S</span> SK <span style="background-color: #e0f0ff;">I</span> I <span style="background-color: #e0f0ff;">H</span> T <span style="background-color: #e0f0ff;">Y</span> I <span style="background-color: #e0f0ff;">K</span> E <span style="background-color: #e0f0ff;">K</span> H <span style="background-color: #e0f0ff;">H</span> N <span style="background-color: #e0f0ff;">E</span> IT <span style="background-color: #e0f0ff;">F</span> G <span style="background-color: #e0f0ff;">D</span> K <span style="background-color: #e0f0ff;">S</span> R <span style="background-color: #e0f0ff;">V</span> G <span style="background-color: #e0f0ff;">R</span> G <span style="background-color: #e0f0ff;">G</span>	238
Consensus	trkapedssgi wdndslaglla elkadll llsdvdgly gppsep sk ihtyikekhqg eittfg dksrvgrgg fg	
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BAA19916	<span style="background-color: #e0f0ff;">MTAK</span> V <span style="background-color: #e0f0ff;">KAA</span> AVL <span style="background-color: #e0f0ff;">S</span> NS <span style="background-color: #e0f0ff;">G</span> T <span style="background-color: #e0f0ff;">P</span> V <span style="background-color: #e0f0ff;">V</span> IT <span style="background-color: #e0f0ff;">S</span> G <span style="background-color: #e0f0ff;">F</span> EN <span style="background-color: #e0f0ff;">R</span> S <span style="background-color: #e0f0ff;">I</span> L <span style="background-color: #e0f0ff;">K</span> V <span style="background-color: #e0f0ff;">L</span> H <span style="background-color: #e0f0ff;">G</span> E <span style="background-color: #e0f0ff;">K</span> I <span style="background-color: #e0f0ff;">G</span> T <span style="background-color: #e0f0ff;">L</span> F <span style="background-color: #e0f0ff;">H</span> K <span style="background-color: #e0f0ff;">N</span> A <span style="background-color: #e0f0ff;">L</span> W <span style="background-color: #e0f0ff;">E</span> S <span style="background-color: #e0f0ff;">S</span> K <span style="background-color: #e0f0ff;">D</span> V <span style="background-color: #e0f0ff;">S</span> T <span style="background-color: #e0f0ff;">R</span> E <span style="background-color: #e0f0ff;">M</span> A <span style="background-color: #e0f0ff;">V</span> A <span style="background-color: #e0f0ff;">A</span> R <span style="background-color: #e0f0ff;">D</span> C <span style="background-color: #e0f0ff;">S</span> R <span style="background-color: #e0f0ff;">H</span> L <span style="background-color: #e0f0ff;">Q</span> N <span style="background-color: #e0f0ff;">L</span> S <span style="background-color: #e0f0ff;">S</span> E <span style="background-color: #e0f0ff;">E</span> R <span style="background-color: #e0f0ff;">K</span>	318
AA35536	<span style="background-color: #e0f0ff;">MTAK</span> V <span style="background-color: #e0f0ff;">QAA</span> V <span style="background-color: #e0f0ff;">V</span> W <span style="background-color: #e0f0ff;">A</span> ST <span style="background-color: #e0f0ff;">G</span> V <span style="background-color: #e0f0ff;">P</span> V <span style="background-color: #e0f0ff;">V</span> IT <span style="background-color: #e0f0ff;">S</span> G <span style="background-color: #e0f0ff;">C</span> A <span style="background-color: #e0f0ff;">S</span> Q <span style="background-color: #e0f0ff;">S</span> L <span style="background-color: #e0f0ff;">V</span> K <span style="background-color: #e0f0ff;">V</span> L <span style="background-color: #e0f0ff;">R</span> G <span style="background-color: #e0f0ff;">E</span> K <span style="background-color: #e0f0ff;">I</span> G <span style="background-color: #e0f0ff;">T</span> L <span style="background-color: #e0f0ff;">F</span> H <span style="background-color: #e0f0ff;">K</span> N <span style="background-color: #e0f0ff;">A</span> L <span style="background-color: #e0f0ff;">W</span> E <span style="background-color: #e0f0ff;">P</span> S <span style="background-color: #e0f0ff;">K</span> E <span style="background-color: #e0f0ff;">T</span> S <span style="background-color: #e0f0ff;">V</span> R <span style="background-color: #e0f0ff;">E</span> M <span style="background-color: #e0f0ff;">A</span> V <span style="background-color: #e0f0ff;">A</span> A <span style="background-color: #e0f0ff;">R</span> D <span style="background-color: #e0f0ff;">C</span> S <span style="background-color: #e0f0ff;">R</span> R <span style="background-color: #e0f0ff;">L</span> Q <span style="background-color: #e0f0ff;">N</span> L <span style="background-color: #e0f0ff;">S</span> S <span style="background-color: #e0f0ff;">E</span> R <span style="background-color: #e0f0ff;">K</span>	318
BAD97364	<span style="background-color: #e0f0ff;">MTAK</span> V <span style="background-color: #e0f0ff;">QAA</span> V <span style="background-color: #e0f0ff;">V</span> W <span style="background-color: #e0f0ff;">A</span> AT <span style="background-color: #e0f0ff;">G</span> V <span style="background-color: #e0f0ff;">P</span> V <span style="background-color: #e0f0ff;">V</span> IT <span style="background-color: #e0f0ff;">S</span> G <span style="background-color: #e0f0ff;">C</span> A <span style="background-color: #e0f0ff;">S</span> Q <span style="background-color: #e0f0ff;">S</span> L <span style="background-color: #e0f0ff;">V</span> K <span style="background-color: #e0f0ff;">V</span> L <span style="background-color: #e0f0ff;">R</span> G <span style="background-color: #e0f0ff;">E</span> K <span style="background-color: #e0f0ff;">I</span> G <span style="background-color: #e0f0ff;">T</span> L <span style="background-color: #e0f0ff;">F</span> H <span style="background-color: #e0f0ff;">K</span> N <span style="background-color: #e0f0ff;">A</span> L <span style="background-color: #e0f0ff;">W</span> E <span style="background-color: #e0f0ff;">P</span> S <span style="background-color: #e0f0ff;">K</span> E <span style="background-color: #e0f0ff;">T</span> S <span style="background-color: #e0f0ff;">V</span> R <span style="background-color: #e0f0ff;">E</span> M <span style="background-color: #e0f0ff;">A</span> V <span style="background-color: #e0f0ff;">A</span> A <span style="background-color: #e0f0ff;">R</span> D <span style="background-color: #e0f0ff;">C</span> P <span style="background-color: #e0f0ff;">R</span> H <span style="background-color: #e0f0ff;">L</span> Q <span style="background-color: #e0f0ff;">N</span> L <span style="background-color: #e0f0ff;">S</span> S <span style="background-color: #e0f0ff;">E</span> R <span style="background-color: #e0f0ff;">K</span>	318
ABM30223	<span style="background-color: #e0f0ff;">S</span> Q <span style="background-color: #e0f0ff;">L</span> K <span style="background-color: #e0f0ff;">R</span> R <span style="background-color: #e0f0ff;">L</span> L <span style="background-color: #e0f0ff;">S</span> W <span style="background-color: #e0f0ff;">L</span> Q <span style="background-color: #e0f0ff;">T</span> V <span style="background-color: #e0f0ff;">A</span> R <span style="background-color: #e0f0ff;">L</span> L <span style="background-color: #e0f0ff;">L</span> Q <span style="background-color: #e0f0ff;">V</span> D <span style="background-color: #e0f0ff;">F</span> A <span style="background-color: #e0f0ff;">S</span> Q <span style="background-color: #e0f0ff;">S</span> I <span style="background-color: #e0f0ff;">L</span> R <span style="background-color: #e0f0ff;">V</span> L <span style="background-color: #e0f0ff;">Q</span> G <span style="background-color: #e0f0ff;">E</span> K <span style="background-color: #e0f0ff;">I</span> G <span style="background-color: #e0f0ff;">T</span> L <span style="background-color: #e0f0ff;">F</span> H <span style="background-color: #e0f0ff;">K</span> D <span style="background-color: #e0f0ff;">A</span> S <span style="background-color: #e0f0ff;">L</span> W <span style="background-color: #e0f0ff;">E</span> P <span style="background-color: #e0f0ff;">S</span> K <span style="background-color: #e0f0ff;">D</span> V <span style="background-color: #e0f0ff;">S</span> A <span style="background-color: #e0f0ff;">R</span> E <span style="background-color: #e0f0ff;">M</span> A <span style="background-color: #e0f0ff;">V</span> A <span style="background-color: #e0f0ff;">A</span> R <span style="background-color: #e0f0ff;">E</span> C <span style="background-color: #e0f0ff;">S</span> R <span style="background-color: #e0f0ff;">L</span> Q <span style="background-color: #e0f0ff;">N</span> L <span style="background-color: #e0f0ff;">S</span> S <span style="background-color: #e0f0ff;">D</span> E <span style="background-color: #e0f0ff;">R</span> K	320
ABS32296	<span style="background-color: #e0f0ff;">MTAK</span> V <span style="background-color: #e0f0ff;">KAA</span> F <span style="background-color: #e0f0ff;">V</span> A <span style="background-color: #e0f0ff;">S</span> NS <span style="background-color: #e0f0ff;">G</span> T <span style="background-color: #e0f0ff;">P</span> V <span style="background-color: #e0f0ff;">V</span> IT <span style="background-color: #e0f0ff;">S</span> G <span style="background-color: #e0f0ff;">F</span> A <span style="background-color: #e0f0ff;">S</span> Q <span style="background-color: #e0f0ff;">S</span> I <span style="background-color: #e0f0ff;">L</span> R <span style="background-color: #e0f0ff;">V</span> L <span style="background-color: #e0f0ff;">Q</span> G <span style="background-color: #e0f0ff;">E</span> K <span style="background-color: #e0f0ff;">I</span> G <span style="background-color: #e0f0ff;">T</span> L <span style="background-color: #e0f0ff;">F</span> H <span style="background-color: #e0f0ff;">K</span> D <span style="background-color: #e0f0ff;">A</span> S <span style="background-color: #e0f0ff;">L</span> W <span style="background-color: #e0f0ff;">E</span> P <span style="background-color: #e0f0ff;">S</span> K <span style="background-color: #e0f0ff;">D</span> V <span style="background-color: #e0f0ff;">S</span> A <span style="background-color: #e0f0ff;">R</span> E <span style="background-color: #e0f0ff;">M</span> A <span style="background-color: #e0f0ff;">V</span> A <span style="background-color: #e0f0ff;">A</span> R <span style="background-color: #e0f0ff;">E</span> C <span style="background-color: #e0f0ff;">S</span> R <span style="background-color: #e0f0ff;">L</span> Q <span style="background-color: #e0f0ff;">N</span> L <span style="background-color: #e0f0ff;">S</span> S <span style="background-color: #e0f0ff;">D</span> E <span style="background-color: #e0f0ff;">R</span> K	318
Consensus	k vkaavlasns gtpvvitsgf enr s ilkvl hgekigtlfhknal wes skdvs tremava ardc srhlqnlss eerk	
AAS89034	<span style="background-color: #e0f0ff;">K</span> ILL <span style="background-color: #e0f0ff;">D</span> V <span style="background-color: #e0f0ff;">A</span> D <span style="background-color: #e0f0ff;">A</span> L <span style="background-color: #e0f0ff;">E</span> AN <span style="background-color: #e0f0ff;">E</span> D <span style="background-color: #e0f0ff;">L</span> I <span style="background-color: #e0f0ff;">R</span> S <span style="background-color: #e0f0ff;">E</span> NE <span style="background-color: #e0f0ff;">A</span> D <span style="background-color: #e0f0ff;">V</span> AA <span style="background-color: #e0f0ff;">Q</span> V <span style="background-color: #e0f0ff;">A</span> G <span style="background-color: #e0f0ff;">Y</span> E <span style="background-color: #e0f0ff;">K</span> P <span style="background-color: #e0f0ff;">L</span> VAR <span style="background-color: #e0f0ff;">L</span> T <span style="background-color: #e0f0ff;">I</span> K <span style="background-color: #e0f0ff;">P</span> G <span style="background-color: #e0f0ff;">K</span> I <span style="background-color: #e0f0ff;">A</span> S <span style="background-color: #e0f0ff;">L</span> A <span style="background-color: #e0f0ff;">K</span> S <span style="background-color: #e0f0ff;">I</span> R <span style="background-color: #e0f0ff;">T</span> L <span style="background-color: #e0f0ff;">A</span> ME <span style="background-color: #e0f0ff;">D</span> P <span style="background-color: #e0f0ff;">I</span> N <span style="background-color: #e0f0ff;">Q</span> I <span style="background-color: #e0f0ff;">L</span> K <span style="background-color: #e0f0ff;">K</span> TE <span style="background-color: #e0f0ff;">V</span> A <span style="background-color: #e0f0ff;">D</span> D <span style="background-color: #e0f0ff;">L</span> V <span style="background-color: #e0f0ff;">L</span>	398
BAA19916	<span style="background-color: #e0f0ff;">K</span> ILL <span style="background-color: #e0f0ff;">D</span> V <span style="background-color: #e0f0ff;">A</span> D <span style="background-color: #e0f0ff;">A</span> L <span style="background-color: #e0f0ff;">E</span> AN <span style="background-color: #e0f0ff;">E</span> D <span style="background-color: #e0f0ff;">L</span> I <span style="background-color: #e0f0ff;">R</span> S <span style="background-color: #e0f0ff;">E</span> NE <span style="background-color: #e0f0ff;">A</span> D <span style="background-color: #e0f0ff;">V</span> AA <span style="background-color: #e0f0ff;">Q</span> V <span style="background-color: #e0f0ff;">A</span> G <span style="background-color: #e0f0ff;">Y</span> E <span style="background-color: #e0f0ff;">K</span> P <span style="background-color: #e0f0ff;">L</span> VAR <span style="background-color: #e0f0ff;">L</span> T <span style="background-color: #e0f0ff;">I</span> K <span style="background-color: #e0f0ff;">P</span> G <span style="background-color: #e0f0ff;">K</span> I <span style="background-color: #e0f0ff;">A</span> S <span style="background-color: #e0f0ff;">L</span> A <span style="background-color: #e0f0ff;">K</span> S <span style="background-color: #e0f0ff;">I</span> R <span style="background-color: #e0f0ff;">T</span> L <span style="background-color: #e0f0ff;">A</span> ME <span style="background-color: #e0f0ff;">D</span> P <span style="background-color: #e0f0ff;">I</span> N <span style="background-color: #e0f0ff;">Q</span> I <span style="background-color: #e0f0ff;">L</span> K <span style="background-color: #e0f0ff;">K</span> TE <span style="background-color: #e0f0ff;">V</span> A <span style="background-color: #e0f0ff;">D</span> D <span style="background-color: #e0f0ff;">L</span> V <span style="background-color: #e0f0ff;">L</span>	398
AA35536	<span style="background-color: #e0f0ff;">K</span> ILL <span style="background-color: #e0f0ff;">D</span> V <span style="background-color: #e0f0ff;">A</span> D <span style="background-color: #e0f0ff;">A</span> L <span style="background-color: #e0f0ff;">E</span> AN <span style="background-color: #e0f0ff;">E</span> D <span style="background-color: #e0f0ff;">L</span> I <span style="background-color: #e0f0ff;">R</span> S <span style="background-color: #e0f0ff;">E</span> NE <span style="background-color: #e0f0ff;">A</span> D <span style="background-color: #e0f0ff;">L</span> AA <span style="background-color: #e0f0ff;">A</span> H <span style="background-color: #e0f0ff;">E</span> A <span style="background-color: #e0f0ff;">G</span> Y <span style="background-color: #e0f0ff;">E</span> S <span style="background-color: #e0f0ff;">A</span> L <span style="background-color: #e0f0ff;">V</span> S <span style="background-color: #e0f0ff;">R</span> L <span style="background-color: #e0f0ff;">T</span> L <span style="background-color: #e0f0ff;">K</span> P <span style="background-color: #e0f0ff;">G</span> K <span style="background-color: #e0f0ff;">I</span> A <span style="background-color: #e0f0ff;">S</span> L <span style="background-color: #e0f0ff;">A</span> K <span style="background-color: #e0f0ff;">S</span> V <span style="background-color: #e0f0ff;">R</span> T <span style="background-color: #e0f0ff;">L</span> A <span style="background-color: #e0f0ff;">N</span> E <span style="background-color: #e0f0ff;">D</span> P <span style="background-color: #e0f0ff;">I</span> N <span style="background-color: #e0f0ff;">E</span> I <span style="background-color: #e0f0ff;">L</span> K <span style="background-color: #e0f0ff;">R</span> T <span style="background-color: #e0f0ff;">E</span> V <span style="background-color: #e0f0ff;">A</span> D <span style="background-color: #e0f0ff;">G</span> L <span style="background-color: #e0f0ff;">V</span> L	398
BAD97364	<span style="background-color: #e0f0ff;">K</span> ILL	

(*Triticum aestivum*); and ABM30223, ABS32296 (*Saccharum officinarum*).

### Sugarcane ScP5CS contains domains homologous to *E. coli* *proA* and *proBA* proteins

The ScP5CS clones efficiently complemented *E. coli*, *proA* and *proBA*, but not *proC*, the predicted two domains showed high homology to the *E. coli* *proBA* and *proA* proteins, and the recombinant ScP5CS protein showed GSA-dependent  $\gamma$ -GK enzyme activity. High homology was also observed to the bacterial and yeast  $\gamma$ -glutamyl kinase at the N-terminus and to the bacterial  $\gamma$ -glutamyl phosphate reductase at the C-terminus of the ScP5CS protein. Searching in GenBank resulted in the conserved domain of the deduced amino acid sequences of sugarcane ScP5CS, which is shown in Figure 2.

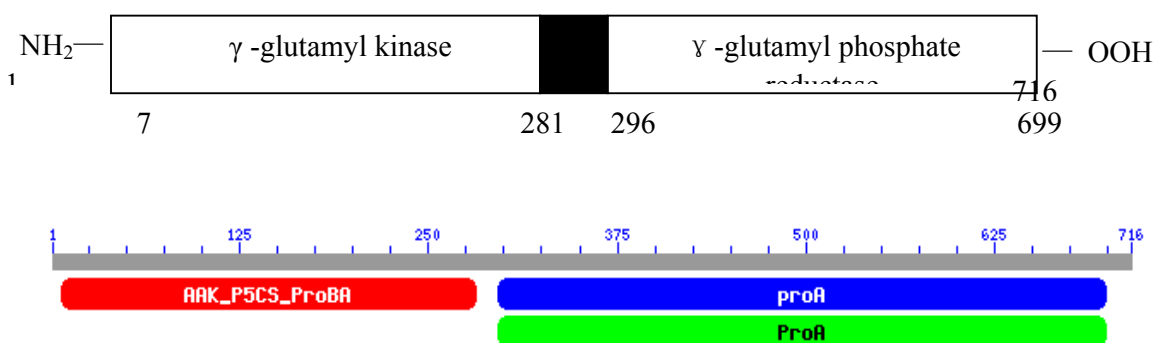


Fig. 2—Conserved domain of the deduced amino acid sequences of sugarcane ScP5CS.

### Discussion

In this study, the cDNA and corresponding gene of ScP5CS encoding the  $\Delta^1$ -pyrroline-5-carboxylate synthetase from sugarcane variety ROC 22 was successfully isolated and the features of the encoded polypeptide characterised. Comparing the sequence of ScP5CS with that of sugarcane P5CS reported in GenBank, the nucleotide acid sequences showed high identity (98%), but the deduced amino acid homology was only 92%. The alignment of ScP5CS with all the presently available similar proteins in GenBank revealed two enzymatic domains corresponding to bacterial and yeast  $\gamma$ -glutamyl kinase and to bacterial  $\gamma$ -glutamyl phosphate reductase. The two residues in the positions 125 and 128, which were aspartate (Asp, D) and phenylalanine (Phe, F) respectively, were conserved in sugarcane ScP5CS and other plants. Site-directed mutagenesis could indicate whether they are implicated in the feedback inhibition by proline.

Amino acid sequence analysis revealed the identical sequences between the ScP5CS and graminaceous plants. There were more differences in Glu-5-kinase domain from the deduced amino acid of the reported sugarcane P5CS (ABM30223), but less for the P5CSs from rice (*Oryza sativa*) and wheat (*Triticum aestivum*). Different molecular structures would lead to different functions for the ScP5CSs. Presently, further functional analysis of this gene is being studied. Characterisation of events underlying P5CS gene expression is currently under investigation, which could provide insights into the role of proline and stress-related changes in gene expression in abiotic stress tolerance of sugarcane.

### Acknowledgements

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#### REFERENCES

- Chiang, H.H. and Dandekar, A.M.** (1995). Regulation of accumulation in *Arabidopsis thaliana* during development and in response to desiccation. *Plant Cell Environment*, 18: 1280–1290.
- Delauney, A.J. and Verma, D.P.S.** (1993). Proline biosynthesis osmoregulation in plants. *The Plant Journal*, 4: 215–223.
- Hare, P.D. and Cress, W.A.** (1997). Metabolic implications of stress-induced proline accumulation in plants. *Plant Growth Regulation*, 21: 79–102.
- Hare, P.D., Cress, W.A. and van Staden, J.** (1998). Dissecting the roles of osmolyte accumulation during stress. *Plant Cell and Environment*, 21: 535–553.
- Hu, C.A.A., Delauney, A.J. and Verma, D.P.S.** (1992). A bifunctional enzyme ( $\Delta^1$ -pyrroline-5-carboxylate synthetase) catalyses the first two steps in proline biosynthesis in plants. *Pro. Natl. Sci. USA. Plant Biology*, 89: 9354–9358.
- Hu, C.A.A., Lin W.W., Obie C. and Valle, D.** (1999). Molecular enzymology of mammalian  $\Delta^1$ -pyrroline-5-carboxylate synthase. *The Journal of Biological Chemistry*, 274(10) 6754–6762.
- Igarashi, Y., Yoshiba, Y., Sanada Y., Yamaguchi-Shinozaki, K., Wada, K and Shinozaki, K.** (1997). Characterization of the gene for  $\Delta^1$ -pyrroline-5-carboxylate synthetase and correlation between the expression of the gene and salt tolerance in *Oryza sativa L.* *Plant Molecular Biology*, 33: 857–865.
- Kishor, K.P.B., Hong, Z., Miao, C.-H., Hu, C.-A. A. and Verma, D.P.S.** (1995). Overexpression of  $\Delta^1$ -pyrroline-5-carboxylate synthetase increases proline production and confers osmotolerance in transgenic plants. *Plant Physiol.*, 108: 1387–1394.
- Ma, L.Q.** (2005). QTL mapping and related gene cloning for salt tolerance in wheat (*Triticum aestivum L.*). China Agricultural University, Ph.D. dissertation, 2005. (in Chinese).
- Savouré, A., Jaoua, S., Hua, X.-J., Ardiles, W., Montagu, M.V. and Verbruggen, N.** (1995). Isolation, characterisation, and chromosomal location of a gene encoding the  $\Delta^1$ -pyrroline-5-carboxylate synthetase in *Arabidopsis thaliana*. *FEBS Letters*, 372: 13–19.
- Wang, G.L. and Fang, H.J.** (2002). *Plant genetic engineering*. Science Press, Beijing, 750–758.
- Yamada, M., Morishita, H., Urano, K., Shiozaki, N., Yamaguchi-Shinozaki, K., Shinozaki, K. and Yoshiba, Y.** (2005). Effects of free proline accumulation in petunias under drought stress. *Journal of Experimental Botany*, 56 (417): 1975–1981.
- Yoshiba, Y., Kiiyosue, T., Katagiri, T., Ueda, H., Mizoguchi, T., Yamaguchi-Shinozaki, K., Wada, K., Harada, Y. and Shinozaki, K.** (1995) Correlation between the induction of a gene for  $\Delta^1$ -pyrroline-5-carboxylate synthetase and the accumulation of proline in *Arabidopsis thaliana* under osmotic stress. *The Plant Journal*, 7: 751–760.

**ISOLEMENT ET CARACTÉRISATION D'UN GÈNE CODANT POUR  
LA  $\Delta^1$  PYRROLINE 5 CARBOXYLATE SYNTHÉTASE CHEZ  
LA CANNE À SUCRE (SACCHARUM SPP. HYBRIDE VAR. ROC22)**

Par

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**MOTS CLÉS: Canne à Sucre,  $\Delta^1$  Gène Pyrroline 5 Carboxylate Synthétase (P5CS), Proline, Stress Hydrique.**

**Résumé**

CET ARTICLE décrit l'isolement et la caractérisation d'un gène codant pour la  $\Delta^1$  pyrroline 5 carboxylate synthétase chez la canne à sucre (*Saccharum* spp. var. hybride ROC22). Des plantules au stade de quatre à cinq feuilles sont soumises à un stress hydrique via l'application d'une solution de 25% polyéthylène glycol (PEG). La séquence ADNc correspondant au gène ScP5CS de la canne à sucre a été isolée par clonage homologue. Cette séquence de 2151 pb, qui contient un ORF (Open Reading Frame) codant 716 acides aminés (numéro d'accèsion Genbank: EU005373), partage un fort pourcentage d'identité (98%) avec les séquences du gène P5CS (EF155655) chez la canne à sucre. Cependant, le pourcentage d'identité entre les deux séquences en acides aminés n'est que de 92%. La protéine déduite possède un site putatif de fixation au NADPH, des domaines leucine conservés, un domaine Glu5-kinase, un domaine GSA-DH ainsi qu'un site de rétro-inhibition. Des différences ont été observées au niveau du domaine Glu-5 kinase de ScP5CS avec les séquences en acides aminés de P5CS (ABM30223). Cependant ces différences sont moins importantes qu'avec les séquences du riz (*Oryza sativa*) et du blé (*Triticum aestivum*). Le gène isolé correspondrait donc à un nouveau gène P5CS chez la canne à sucre.

**AISLAMIENTO Y CARACTERIZACIÓN DE UN GEN QUE CODIFICA LA  
 $\Delta^1$ -PIRROLINA -5- CARBOXILATO SINTETASA EN  
CAÑA DE AZÚCAR (HÍBRIDO DE *SACCHARUM* SPP VAR. ROC22)**

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**PALABRAS CLAVES:** Caña de Azúcar, Gene  $\Delta^1$ -Pirrolina-5- Carboxilato Sintetasa (P5CS), Prolina, Estrés Hídrico.

**Resumen**

LA VARIEDAD de caña ROC22 fue utilizada como el material experimental en el presente estudio. Plantas de dicha variedad que tenían de 4 y 5 hojas fueron sometidas a estrés hídrico aplicándoles una solución de Polietilenglicol (PEG) 6000 al 25%. La secuencia de cADN que codifica para el gen de caña ScP5CS fue aislada por clonación homóloga. La secuencia del gen estaba compuesta de 2151 pb con un marco de lectura abierto de 716 aminoácidos. (Accesión en GenBank número EU005373). Al comparar la secuencia de ScP5CS con la secuencia del gen de caña de azúcar P5CS registrado en Gen Bank, mostró que la secuencia nucleotídica (EF155655) tenía una identidad del 98%, pero la secuencia a nivel de amino ácido fue solo idéntica en un 92%. La proteína contiene un sitio de unión a ATP, un dominio de leucina, un dominio para Glu-5-quinasa, un dominio de union NADPH, un dominio conservado de GSA-DH y un sitio de reacción inhibición. Adicionalmente, se encontraron diferencias a nivel de la secuencia para el dominio Glu-5-quinasa comparadas con las registradas para la secuencia de aminoácidos de P5CS (ABM30223), y menos diferencias al compararlas con la secuencia del gen P5CSs de arroz (*Oryza sativa*) y trigo (*Triticum aestivum*). Por lo tanto creemos que este gen es un nuevo gen de P5CS en caña de azúcar.