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Identification of 14-3-3-like protein in sugarcane (*Saccharum officinarum*)

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Abstract

In a search of the sugarcane expressed sequence tag (SUCEST) database we located three full-length cDNAs (SCCCLR1022D05.g, SCCCRZ1001D02.g and SCBFLR1026E02.g) encoding the 14-3-3 proteins from sugarcane (*Saccharum officinarum*). The encoded proteins were identified based on the clustering of the expressed sequence tags and were shown to encode proteins similar to 14-3-3 proteins of other monocotyledonous plants. Cluster SCCCLR1022D05.g was 99% similar to the maize 14-3-3-like protein (gi|1345587) while cluster SCCCRZ1001D02.g shared 96% and SCBFLR1026E02.g 94% similarity with the 14-3-3 protein of rice (gi|7435022). Although 14-3-3 proteins have been reported to be specific to particular species, tissue or organ from which they were isolated, all three sugarcane clusters were found to be expressed in several tissues.

INTRODUCTION

Sugarcane is one of the most important crops in the world and Brazil accounts for 25% of worldwide production. The genus *Saccharum* is complex and is characterized by high polyploidy and frequent aneuploidy (Sreenivasan *et al*., 1987). The Sugarcane Expressed Sequence Tag (SUCEST) genome project has provided expressed sequence tags (ESTs) that should allow the identification of novel genes with specific expression patterns and allow the assignment of gene function to certain cell types. We have used this database to analyze ESTs encoding sugarcane 14-3-3 proteins.

The 14-3-3 proteins (shortened to 14-3-3s) were originally named on the basis of their electrophoretic mobility in starch gel electrophoresis (Moore and Perez, 1967) and have been reported in mammals (Aitken *et al*., 1992), plants (Hirsch *et al*., 1992; Brandt *et al*., 1992), *Xenopus* (Martens *et al*., 1992), *Drosophila* (Swanson and Ganguly, 1992) *Dictyostelium discoideum* (Knetsch *et al*., 1997) and yeast (van Heusden *et al*., 1992). The 14-3-3s are ubiquitous in eukaryotic cells where they are involved in a variety of biological functions.

The 14-3-3s isolated from mammalian brain tissue are cytosolic (Moore and Perez, 1967) and several brain 14-3-3s have been found in neuronal and synaptic membranes, which suggest more specific involvement in neurotransmission . Some 14-3-3s are associated with cell organelles, e.g. in rat liver a cytosolic 14-3-3 protein, mitochondria stimulating factor (MSF), stimulates the uptake of nuclear encoded precursors into the mitochondria and in *Arabidopsis thaliana* and *Zea mays* 14-3-3s occur in the nuclei (Bihn *et al*., 1997).

The functions of 14-3-3s are much more diverse than originally thought and include protein kinase C regulation (Toker *et al*., 1990), exocytosis (Morgan and Burgoyne, 1992) and ADP-ribosylation of Ras (Fu *et al.*, 1993), as well as being association with transcription factors that regulate gene transcription (de Ventten and Ferl, 1994). Interaction with proteins and protein kinases is a common mechanism by which 14-3-3s influence many functions (Ferl, 1996) and plant 14-3-3s have been found to be part of a transcription factor complex (de Ventten *et al*., 1992) and the association of 14-3-3s with the G-box binding factor suggests that these proteins may play a direct role in transcriptional regulation. While no animals have as yet been shown as having 14-3-3s associated with transcriptional factors, a potential nuclear role has been suggested for 14-3-3s in yeast (rad 24 and rad 25) where they may serve in preventing/regulating DNA damage prior to mitosis (Ford *et al*., 1994).

The activities of several cytosolic enzymes in plant leaves are responsive for changes in photosynthesis. Thus, in addition to providing catabolic/reductant and anabolic/biosynthetic precursors, changes in photosynthesis trigger the release of signals from the chloroplast to control phosphorylation and the activity of cytoplasmic sucrose-phosphate synthase (SPS), nitrate reductase (NR) and phosphoenolpyruvate carboxylase (PEPc) (Foyer *et al*., 1995; MacKintosh, 1998). When photosynthesis is active, SPS, NR and PEPc are activated, and this stimulation presumably increases the flux through sucrose synthesis, and also stimulates the pathways of nitrate assimilation and C4 organic acid synthesis that provide nitrogen and carbon for amino acids. SPS, NR and PEPc are not always regulated in parallel, however, since hormones, water stress, nitrogen and carbon supplies can also modulate the phosphorylation

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and activity of these enzymes. Osmotic stress inactivates NR (Kaiser and Föster, 1989), but a simple phosphorylation-dephosphorylation cycle is not sufficient to explain the control of NR. The inactivation of NR occurs by the phosphorylation of serine-543 (Douglas *et al*., 1995; Su *et al.*, 1996) which is catalyzed by a calcium-dependent (calmodulin-domain) protein kinase (CDPK) or SNF1-related protein kinase (Bachmann *et al*., 1996a*;* Douglas *et al*., 1997; Douglas *et al*., 1998), followed by binding of phosphorylated NR to an NR inhibitor protein (NIP) made up of one or more 14-3-3 proteins (Bachmann *et al*., 1996b; Moorhead *et al.*, 1996) which bind directly to the phosphorylation site of NR. While many proteins have been found to bind to 14-3-3 proteins, plant NR was the first in which a functional 14-3-3s effect (inhibition of phosphorylated NR) was identified in a physiological setting (*i.e.* the response to inhibition of photosynthesis in leaves). In plants, 14-3-3 proteins are also involved in the regulation of the plasma membrane H^+-ATPase (Baunsgaard *et al*., 1998; Jahn *et al*., 1997), and have been found to bind to a number of proteins (including VP1 and EmBP1) which mediate abscisic acid-induced gene expression (Schultz *et al*., 1998). In addition, a bacterially expressed calcium-dependent protein kinase (*Arabidopsis* CPK-1) has been found to bind to, and be activated by, 14-3-3s (Camoni et al., 1998). There is little information on how many 14-3-3-binding targets exist in plants, or on how the phosphorylation-dependent interactions of 14-3-3s with their diverse binding targets are regulated in cells.

In this paper we present the results of our work in which we identified ESTs in the SUCEST database that have a high similarity to 14-3-3 proteins from other monocotyledonous plants.

MATERIAL AND METHODS

The SUCEST project is a cooperative effort between 25 laboratories in the Organization for Nucleotide and Sequence Analysis (ONSA) network supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP). Our group has been involved in data mining for ESTs involved in sugarcane defense mechanisms against pathogens and environmental stress (http://sucest.lad.dcc.unicamp.br/private/mining-reports/BF/BF-mining.htm). The 260,781 sugarcane ESTs sequenced were cDNAs prepared from sugarcane tissues and allocated to different libraries, the libraries being as follows: apical meristem (AM), callus (CL), flowers (FL), lateral buds (LB), leaf roll (LR), leaves LV), root (RT), leaf-root transition zone (RZ), stem bark (SB), seeds (SD), stem internode (ST) and plantlets infected with *Glucoacetobacter diazotroficans* (AD) and *Herbaspirillum rubrisubalbicans* (HR). The cDNAs were prepared from poly adenine polyA+ mRNA using the SuperScript plasmid system kit (Gibco-BRL USA) or the ZAP-cDNA synthesis kit (Stratagene USA). Double-stranded cDNA was fractionated and fragments > 1 kb were used. Sequencing was done using BigDye terminators on an ABI Prism 377 DNA sequencer (Perkin Elmer USA).

The EST clusters were built by alignment using the CAP3 program. The search for 14-3-3 proteins was done using a stringent basic local alignment search tool (BLASP) threshold value of $E < 10^{-143}$. The 14-3-3 protein from the National Center for Biotechnology Information (NCBI) was used as a driver for analysis and aligned by TBLASTN program against the sugarcane EST clusters (http://sucest.lad.dcc.unicamp.br/cgi-bin/prod/blast/form_

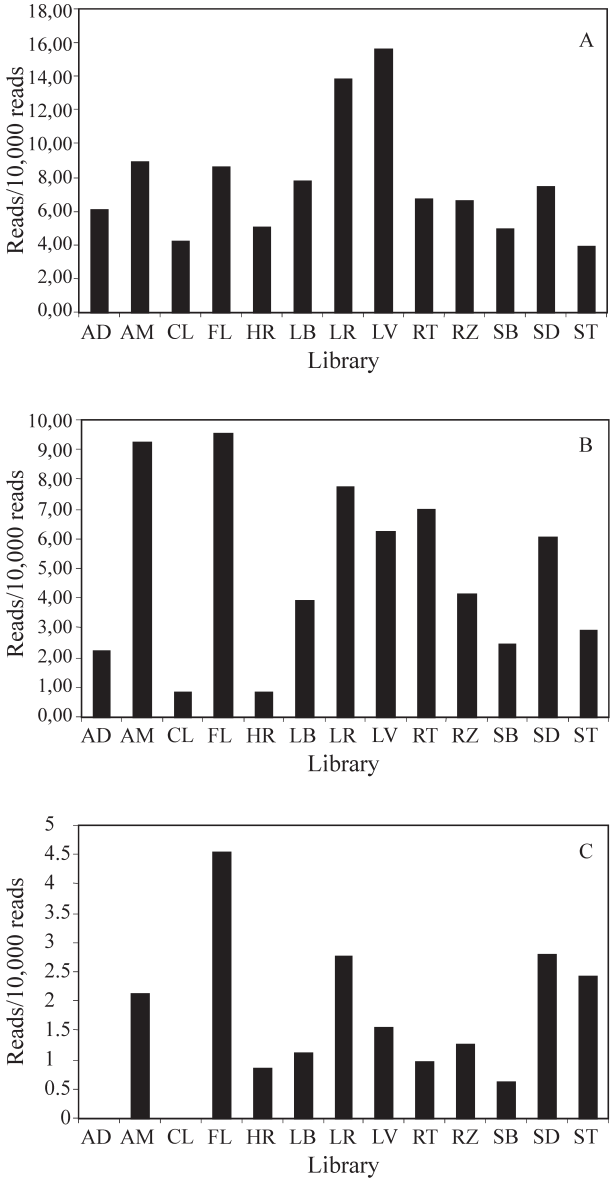

Figure 1 - Percentage of sugarcane cDNA reads per library present in three clusters of 14-3-3 protein. A = SCCCLR1022D05.g, B = SCCCRZ1001D02.g, C = SCBFLR1026E02.g. Libraries, AD = plantlets infected with *Glucoacetobacter diazotroficans*, AM = apical meristem, CL = callus, FL = flower, HR = plantlets infected with *Herbaspirillum rubrisubalbicans*, LB = lateral Bud, LR = leaf roll, LV = leaves, RT = root, RZ = leaf-root transition zone, SB = stem bark, SD = seeds, ST = stem (internode).

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SCBFLR1026E02.g          ------MSREENVYMAKLAEQAERYEEMVEYMEKVAKTVD----VEELTVEERNLLSVAY
SCCCRZ1001D02.g          ------MSREENVYMAKLAEQAERYEEMVEYMEKVAKTVD----VEELTVEERNLLSVAY
GF14-c O. sativa         ------MSREENVYMAKLAEQAERYEEMVEYMEKVAKTVD----VEELTVEERNLLSVAY
SCCCLR1022D05.g          -MASAELSREENVYMAKLAEQAERYEEMVEFMEKVAKTVD----SEELTVEERNLLSVAY
GF14-6 Z. mays           -MASAELSREENVYMAKLAEQAERYEEMVEFMEKVAKTVD----SEELTVEERNLLSVAY
GF14-12 Z. mays          -MASAELSREENVYMAKLAEQAERYEEMVEFMEKVAKTVD----SEELTVEERNLLSVAY
GF14-b O. sativa         MSAQAELSREENVYMAKLAEQAERYEEMVEFMEKVAKTVD----SEELTVEERNLLSVAY
14-3-3 H. vulgare        MSAPGELSREENVYMAKLAEQAERYEEMVEFMEKVAKTVD----SEELTVEERNLLSVAY
14-3-3 O. sativa         MSQPAELSREENVYMAKLAEQAERYEEMVEFMEKVAKTVD----SEELTVEERNLLSVAY
14-3-3b H. vulgare       MAQPAELSREENVYMAKLAEQAERYEEMVEFMEKVAKTVD----SEELTVEERNLLSVAY
GF14 F. agrestis         -MSPAEPSREENVYMAKLAEQAERYEEMVEFMEKVARTVD----TEELTVEERNLLSVAY
14-3-3 L. longiflorum    -MSPAEPSREENVYMAKLAEQAERYEEMVEFMEKVARTVD----TEELTVEERNLLSVAY
TaWIN1 T. aestivum       -MSPAEPTRDESVYMAKLAEQAERYEEMVEFMERVAKATGGAGPGEELSVEERNLLSVAY
GF14-d O. sativa         -MSPAEPTREESVYKAKLAEQAERYEEMVEYMERVARAAGGASGGEELTVEERNLLSVAY

SCBFLR1026E02.g          KNVIGARRASWRIVSSIEQKEESRKNEEHVAQIKEYRGKIEAELSNICDGILKLLDSHLV
SCCCRZ1001D02.g          KNVIGARRASWRIVSSIEQKEESRKNEEHVNLIKEYRGKIEAELSNICDGILKLLDSHLV
GF14-c O. sativa         KNVIGARRASWRIVSSIEQKEEGRGNEEHVTLIKEYRGKIEAELSKICDGILKLLDSHLV
SCCCLR1022D05.g          KNVIGARRASWRIISSIEQKEEGRGNEDRVTLIKDYRGKIETELTKICDGILKLLESHLV
GF14-6 Z. mays           KNVIGARRASWRIISSIEQKEEGRGNEDRVTLIKDYRGKIETELTKICDGILKLLETHLV
GF14-12 Z. mays          KNVIGARRASWRIISSIEQKEEGRGNEDRVTLIKDYRGKIETELTKICDGILKLLESHLV
GF14-b O. sativa         KNVIGARRASWRIISSIEQKEESRGNEDRVTLIKDYRGKIETELTKICDGILKLLESHLV
14-3-3 H. vulgare        KNVIGARRASWRIISSIEQKEESRGNEDRVTLIKEYRGKIETELSKICDGILKLLETHLV
14-3-3 O. sativa         KNVIGARRASWRIISSIEQKEESRGNEDRCTLIKEYRGKIETELSKICDGILKLLDSHLV
14-3-3b H. vulgare       KNVIGARRASWRIISSIEQKEESRGNEDRVTLIKDYRGKIEVELTKICDGILKLLDSHLV
GF14 F. agrestis         KNVIGARRASWRIISSIEQKEESRGNEDHVALIKDYRGKIEAELSKICDGILKLLDSHLV
14-3-3 L. longiflorum    KNVIGARRASWRIISSIEQKEESRGNEDHVALIKDYRGKIEAELSKICDGILKLLDSHLV
TaWIN1 T. aestivum       KNVIGARRASWRIISSIEQKEEGRGNDAHAATIRSYRSKIEAELAKICDGILALLDSHLV
GF14-d O. sativa         KNVIGARRASWRIISSIEQKEEGRGNDAHAATIRSYRGKIEAELARICDGILALLDSHLV

SCBFLR1026E02.g          PSSTAAESKVFYLKMKGDYHRYLAEFKTGTERKESAESTMVAYKAAQDIALAELAPTHPI
SCCCRZ1001D02.g          PSSTAAESKVFYLKMKGDYHRYLAEFKTGAERKESAESTMVAYKAAQDIALAELAPTHPI
GF14-c O. sativa         PSSTAAESKVFYLKMKGDYHRYLAEFKTGAERKEAAESTMVAYKAAQDIALADLAPTHPI
SCCCLR1022D05.g          PSSTAPESKVFYLKMKGDYYRYLAEFKTGAERKDAAENTMVAYKAAQDIALAELAPTHPI
GF14-6 Z. mays           PSSTAPESKVFYLKMKGDYYRYLAEFKTGAERKDAAENTMVAYKAAQDIALAELAPTHPI
GF14-12 Z. mays          PSSTAPESKVFYLKMKGDYYRYLAEFKTGAERKDAAENTMVAYKAAQDIALAELAPTHPI
GF14-b O. sativa         PSSTAPESKVFYLKMKGDYYRYLAEFKTGAERKDAAENTMVAYKAAQDIALAELPPTHPI
14-3-3 H. vulgare        PSSTAPESKVFYLKMKGDYYRYLAEFKSGPERKDAAENTMVAYKAAQDIALAELAPTHPI
14-3-3 O. sativa         PSSTAPESKVFYLKMKGDYYRYLAEFKTGAERKDAAENTMVAYKAAQDIALAELPPTHPI
14-3-3b H. vulgare       PSSTAPESKVFYLKMKGDYYRYLAEFKSGTERKDAAENTMVAYKAAQEIALAELPPTHPI
GF14 F. agrestis         PSSTAAESKVFYLKMKGDYHRYLAEFKSGAERKEAAESTLLAYKSAQDIALAELAPTHPI
14-3-3 L. longiflorum    PSSTAPESKVFYLKMKGDYHRYLAEFKSGAERKEAAESTLLAYKSAQDIALAELAPTHPI
TaWIN1 T. aestivum       PSAGAAESKVFYLKMKGDYHRYLAEFKSGGERKEAAESTMNAYKAAQDIALADLAPTHPI
GF14-d O. sativa         PSAGAAESKVFYLKMKGDYHRYLAEFKSGDERKQAAESTMNAYKAAQDIALADLAPTHPI

SCBFLR1026E02.g          RLGLALNFSVFYYEILNSPDKACNLAKQAFDEAISELDTLGEESYKDSTLIMQLLRDNLT
SCCCRZ1001D02.g          RLGLALNFSVFYYEILNSPDKACNLAKQAFDEAISELDTLGEESYKDSTLIMQLLRDNLT
GF14-c O. sativa         RLGLALNFSVFYYEILNSPDKACNLAKQAFDEAISELDTLGEESYKDSTLIMQLLRDNLT
SCCCLR1022D05.g          RLGLALNFSVFYYEILNSPDRACSLAKQAFDEAISELDTLSEESYKDSTLIMQLLRDNLT
GF14-6 Z. mays           RLGLALNFSVFYYEILNSPDRACSLAKQAFDEAISELDTLSEESYKDSTLIMQLLRDNLT
GF14-12 Z. mays          RLGLALNFSVFYYEILNSPDRACSLAKQAFDEAISELDTLSEESYKDSTLIMQLLHDNLT
GF14-b O. sativa         RLGLALNFSVFYYEILNSPDRACNLAKQAFDEAISELDTLSEESYKDSTLIMQLLRDNLT
14-3-3 H. vulgare        RLGLALNFSVFYYEILNSPDRACNLAKQAFDEAISELDTLSEESYKDSTLIMQLLRDNLT
14-3-3 O. sativa         RLGLALNFSVFYYEILNSPDRACNLAKQAFDEAISELDTLSEESYKDSTLIMQLLRDNLT
14-3-3b H. vulgare       RLGLALNFSVFYYEILNSPDRACDLAKQAFDEAISELDSLSEESYKDSTLIMQLLRDNLT
GF14 F. agrestis         RLGLALNFSVFYYEILNSPDRACNLAKQAFDEAISELDTLGEESYKDSTLIMQLLRDNLT
14-3-3 L. longiflorum    RLGLALNFSVFYYEILNSPDRACNLAKQAFDEAISELDTLGEESYKDSTLIMQLLRDNLT
TaWIN1 T. aestivum       RLGLALNFSVFYYEILNSPDRACNLAKQAFDEAISELDSLGEESYKDSTLIMQLLRDNLT
GF14-d O. sativa         RLGLALNFSVFYYEILNSPDRACNLAKQAFDEAISELDSLGEESYKDSTLIMQLLRDNLT

SCBFLR1026E02.g          LWTSDITEE-GIEEGKEATKG-DAGEGQ
SCCCRZ1001D02.g          LWTSDLTED-GADEGKEASKG-DAGEGQ
GF14-c O. sativa         LWTSDLTED-GGDEVKEASKG-DACEGQ
SCCCLR1022D05.g          LWTSDISED-PAEEIREAPKR-DSSEGQ
GF14-6 Z. mays           LWTSDISED-PAEEIREAPKR-DSSEGQ
GF14-12 Z. mays          LWTSDISED-PAEEIREAPKH-DLSEGQ
GF14-b O. sativa         LWTSDISED-TAEEIREAPKR-DSSEGQ
14-3-3 H. vulgare        LWTSDITEDTAEEEIREAPKH-DSSEGQ
14-3-3 O. sativa         LWTSDISED-AAEEIKEAPKG-ESGDGQ
14-3-3b H. vulgare       LWTSDISED-AAEEMKDAPKG-ESGDGQ
GF14 F. agrestis         LWTSDINEE-AGDEIKEASK---AGEGQ
14-3-3 L. longiflorum    LWTSDINEE-AGDEIKEASK---AVEGQ
TaWIN1 T. aestivum       LWTSDTNED-DVDEIKEAPAPKESGDGQ
GF14-d O. sativa         LWTSDANDD-GGDEIKEAAAPKEPGDQ-
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Figure 2 - Alignment of the deduced amino acid sequences of sugarcane expressed sequence tag (EST) clusters SCCCLR1022D05.g, SCCCRZ1001D02.g and SCBFLR1026E02.g with 14-3-3 proteins from *Oryza sativa* 14-3-3 (gi|7271253), GF14-c (gi|7435022), GF14-b (gi|7435021), and GF14-d (gi|7435023), *Zea mays* GF14-6 (gi|1345587) and GF14-12 (gi|1345588), *Hordeum vulgare* 14-3-3 (gi|7435015) and 14-3-3b (gi|2492487), *Fritillaria agrestis* GF14 (gi|2921512); *Lilium longiflorum* 14-3-3 (gi|12229593), *Triticum aestivum* TaWIN1 (gi|9798603).

maker.pl). Once the corresponding 14-3-3 protein sugarcane cluster was obtained, it was aligned by the BLASTX program to the NCBI protein to check the sequence. Nucleotide and protein sequence alignments were built using the Clustal X (v.1.81) multiple sequence alignment program (Thompson *et al*., 1997).

RESULTS AND DISCUSSION

Three EST clusters (SCCCLR1022D05.g, SCCCRZ 1001D02.g and SCBFLR1026E02.g) similar to 14-3-3 proteins were found in the sugarcane EST genome project database. Cluster SCCCLR1022D05.g was similar to the 14-3-3-like GF14-6 protein of *Zea mays* (gi|1345587), whereas clusters SCCCRZ1001D02.g and SCBFLR1026 E02.g were similar to the 14-3-3-like GF14-c protein of *Oryza sativa* (gi|7435022). The 14-3-3 proteins were expressed in almost all sugarcane libraries constructed from cDNAs from different plant organs (meristem, callus, flower, bud, leaves, root, stem, seed) and from plantlets infected with bacteria (*Glucoacetobacter diazotroficans* and *Herbaspirillum rubrisubalbicans*). The sugarcane 14-3-3 clusters consisted of different cDNAs from different organs (Figure 1a, b and c), whereas 14-3-3 clusters are generally regarded as being specific to a specific tissue or organ (Daugherty *et al*., 1996). Cluster SCCCLR1022D05.g was present in cDNAs from several tissues but showed more reads in leaves and leaf-roll (Figure 1a). The highest read number of cluster SCCCRZ1001D02.g was from flowers and meristems (Figure 1b) while for cluster SCBFLR1026 E02.g the highest read number was from flowers (Figure 1c).

Clusters SCCCLR1022D05.g, SCCCRZ1001D02.g and SCBFLR1026E02 were 1,215 bp, 1,149 bp and 1,274 bp long, respectively. Cluster SCCCLR1022D05.g had a single open reading frame (ORF) of 783 bp encoding a polypeptide of 261 amino acids while clusters SCCCRZ 1001D02.g and SCBFLR1026E02.g both had an ORF of 786 bp encoding 256 amino acid residues (Figure 2). All of the clusters had an in-frame stop codon in the 5' untranslated region and a putative polyadenylation signal in the 3' untranslated region. The sugarcane 14-3-3s were polymorphic in their nucleotide sequences, with the main difference between the sugarcane clusters and the maize and rice 14-3-3 nucleotide sequences occurring in the untranslated regions at the 5 and 3 ends.

The translated sequences of the sugarcane clusters differed mainly at the third nucleotide codon position of the sequences. There were few differences in the deduced sequences of the three clusters (Figure 2). Clusters SCBFLR 1026E02.g and SCCCRZ1001D02.g were more closely related to rice 14-3-3 (gi|7435022) than to cluster SCCCLR 1022D05.g (Figure 3). Cluster SCBFLR1026E02.g had a glutamine at position 82, a threonine at position 150 and an isoleucine at position 126, whereas cluster SCCCRZ1001 D02.g and rice 14-3-3 had leucine, alanine and leucine, respectively, at the same positions (Figure 2). At position 131, cluster SCBFLR1026E02.g had an isoleucine whereas cluster SCCCRZ1001D02.g and rice 14-3-3 had alanine and leucine, respectively. Cluster SCCCLR1022D05.g was more closely related to maize 14-3-3 (gene gi|1345587) than to the other two sugarcane 14-3-3 clusters (Figure 3). Indeed, compared to clusters SCCCRZ1001D02.g and SCBFLR1026E02.g, cluster SCCCLR1022D05.g was five amino acid residues longer (Figure 2). Differences in amino acid composition and sequence could also explain the 14-3-3 isoforms reported by van Heusden *et al*. (1996) for *Arabidopsis thaliana*.

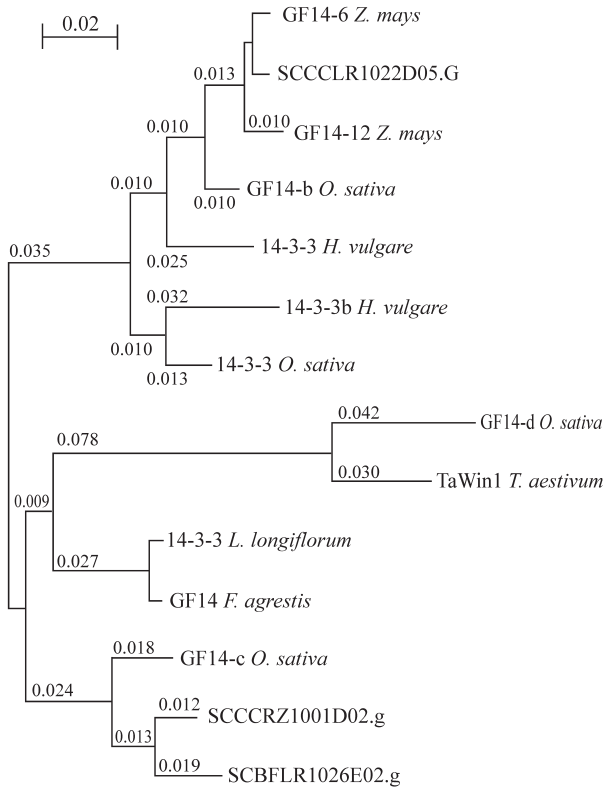

Figure 3 - Dendrogram showing the distance relationships between 14-3-3 amino acid sequences of sugarcane clusters SCCCLR1022D05.g, SCCCRZ1001D02.g and SCBFLR1026E02.g and 14-3-3 proteins of maize, rice and barley. The sequence distance tree was calculated using the neighbor-joining algorithm.

In our study, three full length 14-3-3-like proteins were found in sugarcane, with cluster SCCCLR1022D05.g showing 99% similarity to the 14-3-3-like protein of *Zea mays* (gene gi|1345587) and the deduced amino acid sequence of clusters SCCCRZ1001D02.g and SCBFLR1026 E02.g showing high similarity (96% and 94%, respectively) to the 14-3-3 protein of rice (gene gi|7435022).

RESUMO

Seqüências completas de três cDNAs que codificam proteínas 14-3-3 de cana-de-açúcar (*Saccharum officinarum*) foram encontradas no projeto genoma EST de cana-de-açúcar. As proteínas codificadas por essas seqüên-

cias foram identificadas baseando-se no agrupamento de ESTs de cana-de-açúcar e, três "clusters" (SCCCLR1022 D05.g, SCCCRZ1001D02.g e SCBFLR1026E02.g) foram similares a proteínas 14-3-3 de outras monocotiledôneas. O cluster SCCCLR1022D05.g apresentou similaridade de 99% com a proteína 14-3-3 de milho (gi|1345587) e, os clusters SCCCRZ1001D02.g e SCBFLR1026E02.g foram similares a proteína 14-3-3 de arroz (gi|7435022), 96% e 94%, respectivamente. Embora proteínas 14-3-3 têm sido relatadas como sendo específicas a organismos, tecidos e órgãos, todos os "clusters" de cana-de-açúcar correspondentes a 14-3-3 foram provenientes de cDNAs advindos de diferentes tecidos.

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