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In silico characterization and expression analyses of sugarcane putative sucrose non-fermenting-1 (SNF1) related kinases

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Abstract

Sucrose non-fermenting-1-related protein kinases (SnRKs) may play a major role in regulating gene expression in plant cells. This family of regulatory proteins is represented by sucrose non-fermenting-1 (SNF1) protein kinase in *Saccharomyces cerevisiae,* AMP-activated protein kinases (AMPKs) in mammals and SnRKs in higher plants. The SnRK family has been reorganized into three subfamilies according to the evolutionary relationships of their amino acid sequences. Members of the SnRK subfamily have been identified in several plants. There is evidence that they are involved in the nutritional and/or environmental stress response, although their roles are not yet well understood. We have identified at least 22 sugarcane expressed sequence tag (EST) contigs encoding putative SnRKs. The amino acid sequence alignment of both putative sugarcane SnRKs and known SnRKs revealed a highly conserved N-terminal catalytic domain. Our results indicated that sugarcane has at least one member of each SnRK subfamily. Expression pattern analysis of sugarcane EST-contigs encoding putative SnRKs in 26 selected cDNA libraries from the sugarcane expressed sequence tag SUCEST database has indicated that members of this family are expressed throughout the plant. Members of the same subfamily showed no specific expression patterns, suggesting that their functions are not related to their phylogenic relationships based on N-terminal amino acid sequence phylogenetic relationships.

INTRODUCTION

The SNF1 (sucrose non-fermenting-1) play a major role in regulating gene expression in eukaryotic cells. This family of regulatory proteins is represented by sucrose non-fermenting-1 (SNF1) protein kinase in *Saccharomyces cerevisiae,* AMP-activated protein kinases (AMPKs) in mammals and SnRKs in higher plants. In yeast, SNF1 is essential for the transcription of glucose repressible genes, *i.e.* genes involved in the utilization of alternative carbon sources, gluconeogenesis and respiration (Celenza and Carlson, 1989), as well as peroxisome biogenesis, glycogen biosynthesis, thermo-tolerance and sporulation (Simon *et al.*, 1992; Thompson-Jaeger *et al*., 1991). Yeast SNF1 kinase is activated by phosphorylation in response to glucose deprivation through a so far unknown intracellular signal molecule (Woods *et al*., 1996). Activation of SNF1 has been associated with increases in intracellular AMP and decreases in ATP concentrations, suggesting that these nucleotides might function as signal molecules under conditions of glucose deprivation (Wilson *et al.*, 1996). SNF1 is characterized by an N-terminal serine/threonine kinase domain and a C-terminal regulatory domain (Gancedo, 1998).

In mammalian cells, AMPKs are involved in monitoring cellular energy status by detecting variations in the ratio of ATP to AMP. At high AMP levels, activated AMPKs inactivate enzymes in the anabolic pathway by phosphorylation (Carling *et al.*, 1994). Phosphorylation of other enzymes, such as acetyl-CoA carboxylase, may also lead to the activation of ATP-producing catabolic pathways. Activation of AMPKs in response to stresses, such as high fructose levels, heat shock and oxidative conditions, has also been demonstrated in mammalian cells (Hardie, 1999). AMPKs are heterotrimeric complexes of three subunits. The catalytic α subunit is very similar to the SNF1 and SnRK1 subfamilies, whereas the β subunit shows homology to the SIP1/SIP2/GAL83 subfamily and the γ subunit shows homology to the sucrose non-fermenting-4 (SNF4) family (Woods *et al.*, 1996).

In higher plants, SnRKs have been associated with cellular processes involved in responses to nutritional and environmental stresses that deplete cellular ATP levels (Hardie, 1999; Sudgen *et al.*, 1999). The first plant SnRK was identified in rye endosperm and named RKIN1 (rice homologue of SNF1-endoded protein-serine/threonine kinase) (Alderson *et al.*, 1991). The deduced amino acid sequence of the cDNA encoding RKIN1 showed approximately 48% identity to yeast SNF1 and rat AMPK. Higher levels of similarity (62-64%) between those proteins were found in the N-terminal catalytic domains. SnRKs have also been identified in *Arabidopsis thaliana*, barley, tobacco, wheat, maize, rice and other plant species (Le Guen

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et al. 1992; Muranaka *et al*., 1994 Hannappel *et al*., 1995; Ikeda *et al.*, 1999 and Ohba *et al.*, 2000).

The SnRK family of serine/threonine protein kinases has been reorganized into three subfamilies (SnRK1, SnRK2 and SnRK3), according to the evolutionary relationship of their amino acid sequences, by Halford and Hardie (1998). Members of the SnRK1 subfamily have a highly conserved (*ca.* 62-64% amino acid identity) N-terminal catalytic domain when compared to yeast SNF1 (Halford and Hardie, 1998). Gain of function experiments have shown that members of the SnRK1 subfamily can complement yeast *snf1*-defective mutants, suggesting that the SnRK1 subfamily is also involved in glucose signaling and transcription regulation (Muranaka *et al*., 1994). Members of the SnRK2 subfamily are also very similar (*ca.* 42-45% amino acid identity) to SNF1, mostly in the N-terminal catalytic domain, and have relatively short C-terminal conserved domains when compared to members of the SnRK1 subfamily. Members of the SnRK3 subfamily do not show similarity in the C-terminal domains when compared to members of the SnRK1 and SnRK2 subfamilies (Halford *et al*., 1998).

The roles of SnRKs in plants are not well understood, although it has been reported that the expression of several SnRKs is regulated by hormones and environmental stimuli (Halford and Hardie, 1998). In rice, OsPK4 (*Oryza sativa* protein kinase 4) and OsPK7 (*O. sativa* protein kinase 7) (a SnRK3 subfamily member) are differentially expressed when cells are treated with cytokinins, exposed to high intensity light or deprived of nutrients (Ohba *et al*., 2000), whereas rice PKABA1 (abscisic acid inducible protein kinase) (a SnRK1 subfamily member) is induced by abscisic acid (Hotta *et al*., 1998). Maize ZmPK4 (*Zea mays* protein kinase 4) (a SnRK3 subfamily member) is constitutively expressed in all plant tissues and is induced at low temperatures (Ohba *et al.*, 2000). Koch *et al.* (2000) have suggested that SnRKs may also be involved in controlling carbohydrate metabolism by a glucose sensing mechanism similar to that seen with SNF1. The addition of glucose or sucrose to plant cell suspensions results in decreased expression of genes encoding photosynthetic enzymes and enzymes of the glyoxylate cycle (Krapp *et al.*, 1993; Graham *et al*., 1997), resembling the glucose repression of gene expression observed in yeast. In potato tubers, SnRK1 activity is essential for the expression of the sucrose synthase gene (Purcell *et al.*, 1998). Additionally, Douglas *et al.* (1997) have shown that one of the nitrate reductase kinases in spinach leaves is a member of the SnRK1 subfamily, suggesting that SnRKs have a major role in controlling key enzymes in the biosynthetic pathways of plants.

In order to identify sugarcane expressed sequence tag (EST) contigs encoding putative members of the SnRK family, we searched the SUCEST database for sequences with high similarity to SNF1 using the basic local alignment tool (TBLASTN) of Altschul *et al.* (1990). From the 112 sugarcane EST-contigs similar to SNF1 ($e \leq 10^{-20}$) identified, 22 showed a very conserved N-terminal catalytic domain. The sequences of the conserved N-terminal domain were compared to already described SnRKs and their phylogenetic relationship established. Additionally, the expression profiles of the putative sugarcane SnRKs were determined in 26 different cDNA libraries from the SUCEST database and similarities between their expression profiles determined.

MATERIALS AND METHODS

Identification of putative sugarcane SnRKs

The deduced amino acid sequence of the *S. cerevisiae SNF1* gene (Sucrose Non Fermenting 1; accession number M13971) was used to identify similar EST-contigs in the SUCEST database (http://sucest.lad.dcc.unicamp.br/en). The search was performed using the TBLASTN program with a BLOSUM62 substitution matrix and a cut-off value of $e \leq 10^{-20}$ (Altschul *et al*., 1990). Details on the construction of the cDNA library, sequencing methodology and the procedure used for clustering ESTs into contigs using the CAP3 program (Contig Assembly Program - third generation) (Huang and Madan, 1999) are described elsewhere (Telles and Silva, 2001; Vettore *et al.*, 2001; http://sucest.lad.dcc.unicamp.br/en).

Analyses of sugarcane SnRKs

Amino acid sequences of putative SnRKs were deduced from EST-contig nucleotide consensi using the Sequence Utilities program (http://searchlauncher.bcm.tmc.edu) of Smith *et al*. (1996). The alignment of 22 sugarcane EST-contigs encoding putative members of the SnRK subfamily and several SnRKs from *A. thaliana*, rice, barley and wheat, and their phylogenetic relationships (based on N-terminal amino acid sequences) were performed using AlignX (Vector NTI Program, InforMax, Inc., default parameters).

Expression analysis of putative SnRKs in sugarcane tissues

The expression profiles of the EST-contigs encoding putative SnRKs in 26 cDNA libraries with more than 900 useful reads were determined using hierarchical clustering (Eisen *et al*., 1998). The libraries were prepared from mRNA isolated from different sugarcane tissues at different developmental stages (Table I). For each EST-contig encoding a putative SnRK, the frequency of reads in a selected library was determined and normalized for the total number of useful reads of that library. Hierarchical clustering of the EST-contigs and libraries was performed using an un-centered correlation matrix and the average-linkage method, through the Cluster and Tree View programs (Eisen

et al.,1998). The data matrix was re-ordered according to similarities in the gene expression pattern, and displayed as colored arrays of EST-contigs using a color scale representing the relative number of reads from a specific library for each EST-contig.

Table I - Summary description of the libraries used in this study. For more details, see http://sucest.lad.dcc. unicamp.br/en.

Library	Sumary description
AD1	Plantlets infected with *Glucoacetobacter diazotroficans*
AM1	Apical Meristem, young leaves and Stem of mature sugarcane plants
AM2	Apical Meristem, young leaves and Stem of immature sugarcane plants
CL6	Callus (light/dark and temperature 4-37 °C)
FL1	Flower - 1 cm
FL3	Flower - 5 cm
FL4	Flower - 50 cm
FL5	Flower - 20 cm
FL8	Flower - 10 cm
HR1	Plantlets infected with *Herbaspirillum rubrisubalbicans*
LB1-2	Lateral Bud of plants in different growing conditions
LR1-2	Leaf Roll with different cDNA fragment sizes
LV1	Leaves
RT1, 2 and 3	Root of plants in different growing conditions
RZ1- 2	Leaf-root Transition Zone of mature plants with different cDNA fragment sizes
RZ3	Leaf-root transition Zone of immature plants
SB1	Sten Bark
SD1-2	Seed in different stage of development
ST1-3	Stem - First and fourth internode, respectively

RESULTS AND DISCUSSION

Identification of putative sugarcane SnRKs

Based on amino acid sequence similarity, we identified 112 sugarcane EST-contigs highly similar to SNF1 ($e \leq 10^{-20}$) (Table II). From those ESTs, 22 encoded putative SnRKs with a highly conserved N-terminal catalytic domain also present in the SnRKs of several other plant species (Figure 1). Approximately 14% of these sequences were 200-300 amino acids long, 41% were 301-400, 36% were 401-500 and 9% were longer than 500 amino acids.

Analyses of sugarcane SnRKs

SnRKs have been classified into three subfamilies: SnRK1, SnRK2 and SnRK3, based on amino acid sequence identity and expression patterns (Halford and Hardie, 1998). Comparing the amino acid sequences of sugarcane EST-contigs encoding putative SnRKs with known SnRKs (Halford and Hardie, 1998) we were able to assign our putative sugarcane SnRKs to different subfamilies: three to SnRK1 (two to SnRK1a and one to SnRK1b subfamily), nine to SnRK2 (four to SnRK2a and five to SnRK2b) and ten to SnRK3 (Figure 2). In our phylogenetic analysis, yeast SNF1 and rat AMPK formed a different clade which show a close relationship with the SnRK1 subfamily (Figure 2), similar to what was reported by Halford and Hardie (1998). However, in contrast to these authors, we found that the SnRK1 subfamily was more related to the SnRK3 than to the SnRK2 subfamily. We also found that omitting the sugarcane EST-contigs SCCCLR1068F10 and SCJFLR 1074F04 from the analysis, the phylogenetic relationships between the families was similar to that seen by Halford and Hardie (1998), *i.e.* the SnRK1 subfamily was more re-

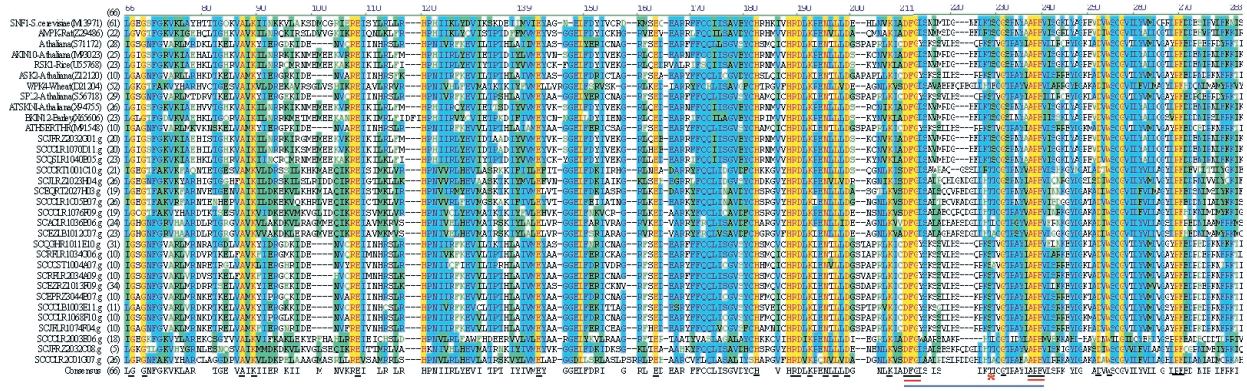

Figure 1 - Alignment of the N-terminal catalytic and T-loop domains of sugarcane EST-contigs encoding putative SnRKs, SNF1, AMPK and other SnRKs. Accession numbers are: SNF1-*S. cerevisiae* (M13971); AMPK-Rat (Z29486); *A. thaliana* (S71172); AKIN10-*A. thaliana* (M93023); RSK1-Rice (U55768); ASK2-*A. thaliana* (Z12120); WPK4-Wheat (D21204); SPL2 - *A. thaliana* (S56718); ATSKINI-*A. thaliana* (X94755); BKIN12 - Barley (X65606); ATHSERTHR (M91548). Amino acids highlighted in yellow are identical in all the proteins studied. Blue represents consensus residues derived from a block of similar residues; green represents consensus residues derived from the occurrence of more than 50% of a single residue at a given position. The conserved T-loop domain is underlined in blue. The conserved T-loop APE and DFG boundary consensi are underlined in red. The putative phosphorylated threonine is indicated by an asterisk (*). Amino acid residues present in all SnRKs are underlined in black.

Table II - EST-contigs with similarity to SNF1 (Acession number M13971) identified using TBLASTN program and the BLOSUM62 substitution matrix.

EST-contigs	Score	E-value	EST-contigs	Score	E value
SCJFRZ2032G01.g	381	e-105	SCACHR1040D12.g	121	1e-27
SCCCLR1070D11.g	380	e-105	SCCCLR1075E10.g	120	4e-27
SCQSLR1040B05.g	358	8e-99	SCCCLR1022H07.g	118	1e-26
SCCCRT1001C10.g	265	1e-70	SCMCCL6052D11.g	118	2e-26
SCJLRZ1023H04.g	262	5e-70	SCVPST1060B01.g	118	2e-26
SCEQRT2027H03.g	254	1e-67	SCJFRZ2007A03.g	117	2e-26
SCCCLR1C05B07.g	252	5e-67	SCVPRT2075A12.g	116	5e-26
SCCCCL5001D11.g	139	2e-66	SCCCRZ2001D07.g	116	5e-26
SCCCLR1076F09.g	236	3e-62	SCCCRT2002G11.g	116	7e-26
SCACLR1036B06.g	234	2e-61	SCEZRZ1014F04.g	116	7e-26
SCUTLR1037E04.g	230	3e-60	SCBGLR1023A01.g	115	1e-25
SCEZLB1012C07.g	229	6e-60	SCCCRZ2C04F05.g	114	2e-25
SCJFRZ2032C08.g	213	3e-55	SCJLRT1014C03.g	114	2e-25
SCCCLR2C01G07.g	200	3e-51	SCJFRZ2011A04.g	114	3e-25
SCQGHR1011E10.g	191	1e-48	SCCCRZ3001D06.g	110	3e-24
SCRFLR1034G06.g	189	8e-48	SCSGLR1084B06.g	110	3e-24
SCSFFL4017G11.g	188	1e-47	SCEZLR1031G10.g	110	3e-24
SCCCST1004A07.g	187	2e-47	SCEPLR1030H10.g	109	5e-24
SCRFLR2034A09.g	187	3e-47	SCBGLR1023A11.g	109	6e-24
SCEZRZ1013F09.g	185	9e-47	SCJFLR1017A05.g	109	6e-24
SCEPRZ3044B07.g	181	2e-45	SCVPRZ2042C11.g	109	6e-24
SCJLRT1023G09.g	180	3e-45	SCCCLR1067H01.g	109	8e-24
SCCCLB1003E11.g	177	3e-44	SCCCRZ2C04G10.g	109	8e-24
SCEPAM2012F04.g	176	3e-44	SCJFRT1008D02.g	108	1e-23
SCMCRT2088D10.g	107	4e-44	SCSGAM2077H04.g	108	1e-23
SCBFSB1046D04.g	175	1e-43	SCSGRT2064G11.g	107	2e-23
SCCCLR1068F10.g	167	2e-41	SCSGFL4031C01.g	107	2e-23
SCEQRT1027E02.g	164	2e-40	SCMCRT2103B04.g	107	2e-23
SCQSSB1055G03.g	164	2e-40	SCQGRT1042H04.g	107	3e-23
SCCCST1006B11.g	92	3e-39	SCSBHR1051C11.g	106	4e-23
SCJFLR1074F04.g	157	3e-38	SCCCRZ1003E02.g	106	6e-23
SCEPRZ1009C10.g	153	3e-37	SCQGLR2017D05.g	106	7e-23
SCEQRT2099H01.g	150	3e-36	SCEQRT1030A09.g	106	7e-23
SCEPRZ1008C06.g	150	4e-36	SCCCRZ2C01D08.g	102	8e-22
SCCCLR2003E06.g	149	5e-36	SCCCLR2C02G01.g	102	8e-22
SCCCLR1C04F08.g	148	9e-36	SCUTAM2008H02.b	101	1e-21
SCCCLB1C03G07.g	146	5e-35	SCQSLR1089G03.g	101	1e-21
SCJLLR1011H04.g	146	2e-35	SCBGLR1117D09.g	101	2e-21
SCEQLB2019B08.g	145	8e-35	SCJFRZ2027G02.g	101	2e-21
SCCCLR1024H03.g	143	4e-34	SCAGRT2041C12.g	100	3e-21
SCEPRZ1009A12.g	143	5e-34	SCSGRT2066D03.g	100	4e-21
SCEQRT2094G10.g	141	2e-33	SCACLR1057B10.g	100	4e-21
SCCCLR1C04E07.g	140	4e-33	SCJFLR1073B02.g	100	6e-21
SCCCRZ1002A06.g	139	5e-33	SCEPRZ3086D11.g	99	9e-21
SCMCLR1122G02.g	137	2e-32	SCEPRZ3045E10.g	99	1e-20
SCEZAD1079D11.g	137	3e-32	SCVPLB1017G09.g	98	2e-20
SCCCLR1079G08.g	134	2e-31	SCVPRT2074E06.g	97	4e-20
SCCCLR1077C07.g	132	6e-31	SCCCLR1072C05.g	97	4e-20
SCEZRZ3019D10.g	132	1e-30	SCRFLR1055G12.g	97	4e-20
SCRULR1020G01.g	131	2e-30	SCCCCL4011A09.g	97	4e-20
SCAGLB2046F06.g	129	5e-30	SCCCRT1001F08.g	97	5e-20
SCMCLR1053D03.g	128	2e-29	SCJLHR1026C05.g	97	5e-20
SCSGAM2075E08.g	124	2e-28	SCJLRT2049D02.g	96	6e-20
SCRLAM1014B03.g	124	2e-28	SCEQRT2030G04.g	96	6e-20
SCAGLR1043A07.g	123	4e-28	SCCCSD1001H06.g	96	8e-20
SCCCLR1C01H12.g	122	1e-27	SCJLRT2049F12.g	96	8e-20

lated to the SnRK2 than to the SnRK3 subfamily. While our data supports the proposition of Halford and Hardie (1998) that SnRKs can be clustered in at least 3 subfamilies, it also suggests that the relationship between SnRKs might vary depending on the members of the data-set.

It has been shown that SNF1 is regulated by phosphorylation and that the phosphorylation site is the threonine-210 residue (Gancedo, 1998). Mutations at the conserved phosphorylation site in SNF1 results in loss of function (Estruch *et al.*, 1992). The mammalian AMPKs are also activated by an upstream protein kinase named AMP-activated protein kinase kinase (AMPKK) by phosphorylation at the threonine-172 residue (Hawley *et al*., 1996). Similarly, SnRKs have also shown to be regulated by phosphorylation (Mackintosh *et al*., 1992). SNF1, AMPKs and SnRKs have a conserved motif at the N-terminal domain delimited by the consensi amino acid sequences DFG (DFG triplets residue) and APE (the "T-loop motif") which contains the serine/threonine phosphorylation site (Sugden *et al*., 1999). Figure 1 shows that the T-loop motif is present in all putative sugarcane SnRKs, suggesting conserved activities. Most of the divergence in the amino acid sequences of sugarcane EST-contigs encoding putative SnRKs was observed in the C-terminal regulatory domains, suggesting that they might be involved in the control of different cellular processes.

Expression analysis of SnRKs in sugarcane tissues

It has been shown that members of SnRK subfamilies are differently regulated in response to environmental stimuli, such as light, nutrient deprivation and cytokinins (Ohba *et al*., 2000), as well as during plant development (Takano *et al*., 1998, Frattini *et al.*, 1999). In rice, the expression of SnRKs (*e.g. O. sativa* kinase – OSK2 and OSK5) is transiently induced in the early stages of seed development (Takano *et al*., 1998), whereas the expression of *REK* (rice endosperm kinase) was induced specifically in leaves and maturing seeds but not in stem and roots (Hotta *et al.*, 1998), suggesting that plant SnRKs might have a role in tissue or organ development. In order to determine the specific expression patterns of the 22 putative sugarcane SnRKs described above, we evaluated their expression profiles in 26 selected cDNA libraries from the SUCEST database using hierarchical clustering (Figure 3). Our data indicate that EST-contigs encoding putative members of sugarcane SnRK subfamilies have no specific expression patterns in the cDNA libraries analyzed, suggesting that

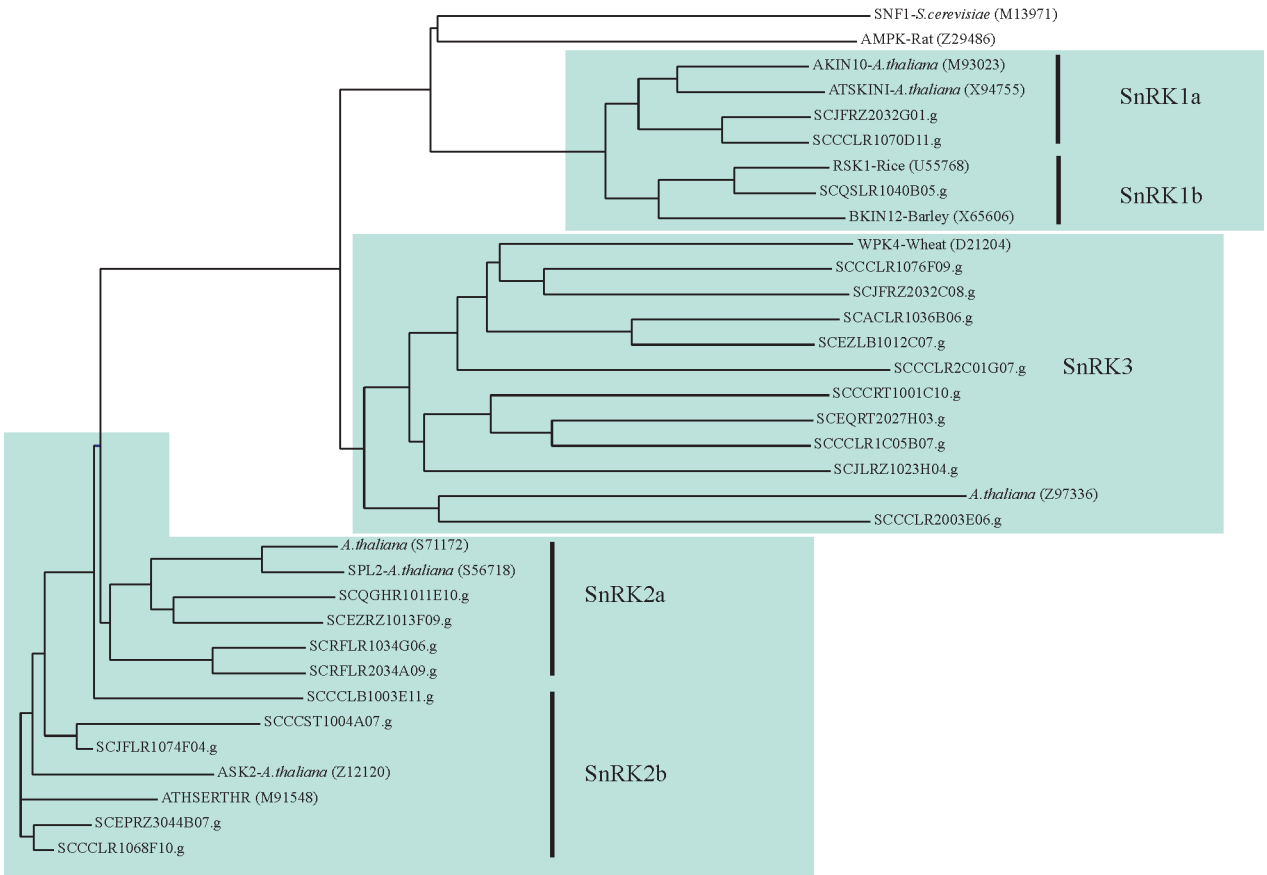

Figure 2 - Phylogenetic relationships between members of the SNF1 family of protein kinases. Alignment of amino acid sequences was performed using AlignX (Vector NTI program). SnRKs subfamilies are boxed in blue. The accession number of previously characterized proteins is shown after their names.

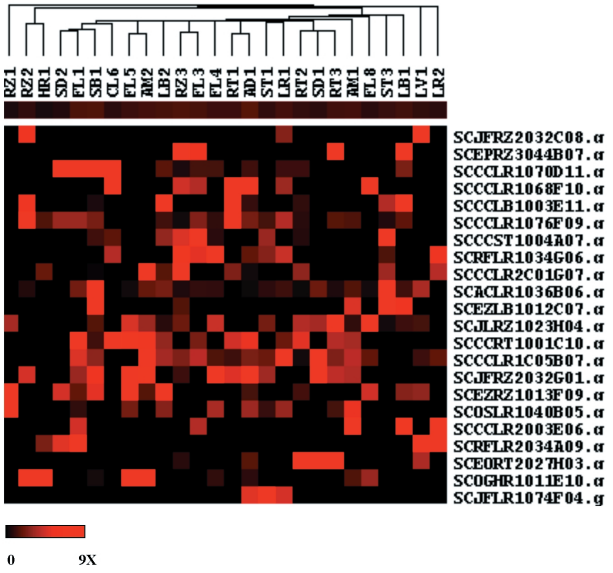

Figure 3 - Expression patterns of putative sugarcane SnRKs in distinct cDNA libraries. The 22 sugarcane EST-contigs encoding putative SnRKs were clustered based on their levels of expression in the selected cDNA libraries. Data represent the relative number of reads from a specific library in each EST-contig per 10,000 reads. Each EST-contig is represented by a single row and each library is represented by a single column. Black means no expression, while increasing intensity to red indicates a 0 to 9-fold increase in the level of expression.

their functions are not related to their phylogenetic relationships based on similarity in their N-terminal amino acid sequences. The presence of at least one EST-contig encoding a putative SnRK in each cDNA library suggests that this kinase family is expressed in all sugarcane tissues. Additionally, the expression profiles of different EST-contigs encoding SnRKs did not show significant correlation. The differential expression of some EST-contigs in libraries from the same tissue, *e.g.* EST-contigs SCCCLR2C01 G07.g in the roots of plants growing under different conditions (RT1 and RT3 libraries) suggests that environmental factors might play important roles in controlling the expression of SnRKs, and that these enzymes might be involved in the response of plants to environmental changes, supporting the view of Ohba *et al.* (2000), although experimental data will be necessary to determine the true role of SnRKs in the development of sugarcane and its response to stresses.

In summary, we have identified 22 sugarcane EST-contigs encoding putative SnRKs, all of which had highly conserved N-terminal catalytic and T-loop mitives. The phylogenetic relationships between sugarcane SnRKs and previously reported SnRKs indicated that the three previously identified subfamilies contain at least one putative sugarcane SnRK. EST-contigs encoding putative SnRKs showed no specific expression pattern in 26 cDNA libraries, and at least one SnRK was expressed in each library. Some EST-contigs showed differential expression in the same tissue at different stages of development and/or under different environmental conditions. Our data suggest that sugarcane SnRKs might be involved in the development of plants or their response to the environment, although experimental evidence will be needed to determining their exact functions.

RESUMO

Quinases de proteínas relacionadas a SNF1 (SnRK) podem desempenhar um papel importante na regulação da expressão gênica em células vegetais. Essa família de proteínas regulatórias é representada pela quinase de proteínas SNF1 (*sucrose non-fermenting –1*) em *Saccharomyces cerevisiae*, AMPKs (quinase de proteínas ativadas por AMP) em células de mamíferos e SnRKs (quinase de proteínas relacionadas a SNF1) em células vegetais. A família de SnRKs foi reorganizada em três subfamílias de acordo com suas relações filogenéticas com base nas seqüências de aminoácidos das proteínas. Membros das subfamílias de SnRKs foram identificados em diversas plantas. Existem evidências mostrando que essa família de proteínas está envolvida em resposta a estresses (nutricional e ambiental), apesar de seu papel não ser totalmente compreendido. Nesse trabalho nós identificamos 22 contíguos de ESTs (*expressed sequence tags*) de cana-de-açúcar codificando SnRKs putativas. O alinhamento das seqüências de aminoácidos das SnRKs putativas de cana-de-açúcar com seqüências de aminoácidos de SnRKs identificadas em outras plantas revelaram um domínio catalítico N-terminal altamente conservado. Além disso, nossos resultados indicaram que em cana-de-açúcar há pelo menos um membro de cada subfamília de SnRKs. Análise do padrão de expressão dos contíguos de EST codificando para SnRK putativas nas 26 bibliotecas selecionadas do banco de dados do Sucest, indicou que membros dessa família de quinases são expressos por toda planta. Membros de cada subfamília não apresentaram padrões de expressão específicos, sugerindo que suas funções não estão correlacionadas com sua relação filogenética, com base nas seqüências de aminoácidos da região N-terminal.

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