Update section

Short communication

Presence of three rice sucrose synthase genes as revealed by cloning and sequencing of cDNA

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Abstract

By sequencing cDNA clones, we have concluded that three distinct sucrose genes are expressed in rice (*Oryza sativa* cv. Tainong 67). When the amino acid sequences deduced from these cDNAs as well as those of known sucrose synthase are compared, the highest divergence is found in the C-termini. The most suitable DNA sequences for use as specific for the mRNA derived from these genes have been suggested.

We concluded from a tracer study using double-labeled sucrose as substrate that the sugar nucleotide synthesized from sucrose and a nucleotide diphosphate such as ADP or UDP via a reaction catalyzed by sucrose synthase (SS; sucrose-UDP glucosyltransferase, EC 2.4.1.13.) could be utilized directly in starch synthesis in maturing rice grains [2, 9]. This conclusion confirms the findings by Chourey and Nelson [5, 6] that the *Sh1* gene of maize codes for an SS (now known as SS1) and its deficiency leads to a shrunk or collapsed phenotype of the kernel due to a reduced level of starch content in the endosperm. From the genetic and biochemical analyses, Chourey and coworkers [3, 4] have shown further that, besides the SS1, maize contains the second isozyme SS2 which is coded by the constitutive

Sus gene [11]. Chourey *et al.* [13, 16] and Heinlein and Starlinger [14] demonstrated that the expression of these two genes had different tissue and cell specificities in the developing maize. By analyzing by chemical and immunological methods, the distribution of SS in different organs of the rice plant at different growth stages, we have also shown that there are tissue- and growth stage-specific partitions of rice SS isozymes [1]. Yang and Russell [17] reported that the promoter sequence of the maize *Ss1* gene in a chimeric recombinant DNA directs phloem cell-specific expression of GUS gene in transgenic tobacco plants. Besides, the differences in the expression of SS genes in response to different types of stress have been reported in several crop plants [10, 12, 14]. All these findings point to the importance of

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RSs1  DLANLVFVCGDHGHNHAKDREEQAEFFKKMYGLIDQYKLKGYIRGISAQMNR
RSs2  ELVNLVVVCGDHGNSKDKKEEQAEFFKKMFDLIEQYNLNGHIRWISAQMNR
RSs3  ELVNLVVVCGDHGKESKDKKEEQAEFFKKMFNLIEQYNLNGHIRWISAQMNR

RSs1  VRNGELYRYICDTKGVFVQPAFYEAFFGLTVIEAMTCGLPTIATSHGGLAE    100
RSs2  VRNGELYRYICDTKGAFFVQPAFYEAFFGLTVVESMTTCGLPTFATAYGGPAE
RSs3  VRNGELYRYICDMRGAFVQPALYEAFFGLTVIEAMTCGLPTFATAYGGPAE

RSs1  IID*GVSGLHIDPYHSDKAADILVNFFEKCKQDSTYWDNISQGGGLQRIYE
RSs2  IIVNGVSGFHIDPYQGDKASALLVEFFEKCQEDPSHWTKISQGGGLQRIEE
RSs3  IIVHGVSGLHIDPYQNDKASRLLEFFEKCQEDPNHWIKISQGGGLQRIEE

RSs1  KYTWKLYSERLMTLTGVYGFWKYVSNLERRETRRYIEMFYALKYRSLASA    200
RSs2  KYTWKLYSERLMTLTGVYGFWKYVSNLERRETRRYLEMLYALKYRTMAST
RSs3  KYTWKLYSERLMTLSGVYGFWKYVTNLDT

RSs1  VPLAV
RSs2  VPLAVEGEPSNK

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Fig. 1. A comparison of C-termini sequences of three rice SS isozymes.

SS isozymes in the physiological function of carbon sink tissues in higher plants.

The rice SS isozymes are very difficult to purify because of their proteolytic susceptibility in the extract and even in a highly purified state [1, 18]. Besides, the best enzyme samples obtained so far are, like the maize enzyme [15], blocked at the N-terminus to resist direct sequence analysis by the Edman procedure. In order to elucidate the primary structures of rice SS isozymes and to look into the physiological roles played by these enzymes, we have cloned and sequenced the rice SS cDNA and genomic DNA. We already know that there is a strong immunological cross-reactivity among all of the known rice SS isozymes and between rice and maize SS [1, 8]. So, we could use the maize SS genomic DNA clones, p17.6 and p21.2, generous gifts of Professor L.C. Hannah of the University of Florida, Gainesville, as probes and their published sequences [19] as references to delineate our results. We report the sequences of cDNA and genomic DNA of rice SS (*RSs*) genes.

Table 1. Amino acid sequence homologies (%) among sucrose synthase isozymes from various plants. Overlapping sequences of rice (*RSs*1, *RSs*2, *RSs*3), wheat (*WSs*1, *WSs*2), maize (*MSh*1, *MSs*2) and potato (*PSs*) sucrose synthases were compared.

	<i>PSs</i>	<i>MSs</i> 2	<i>MSh</i> 1	<i>WSs</i> 2	<i>WSs</i> 1	<i>RSs</i> 3	<i>RSs</i> 2
<i>RSs</i> 1	70	73	87*	76	87	76	80
<i>RSs</i> 2	76	90	81	91	82	90	
<i>RSs</i> 3	74	82	78	91	80		
<i>WSs</i> 1	74	75	91	80			
<i>WSs</i> 2	74	85	79				
<i>MSh</i> 1	71	75					
<i>MSs</i> 2	70						

* Higher homologies are in bold type.

The most important conclusion we have obtained in this study is that there are at least three SS genes, one more than from any other known sources, expressed in the rice. Of all sequenced clones, a full-length cDNA and an assemblage of exons from two overlapping genomic DNA clones had an identical open reading frame that could be translated into the primary structure of a type 2

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RSs2  GHIRWISAQMNRVRNGELYRYICDTKGAFFVQPAFYEAFFGLTVVESMTTCGLPTFAT
RSs3  GHIRWISAQMNRVRNGELYRYICDMRGAFVQPALYEAFFGLTVIEAMTCGLPTFAT
MSh1  GHIRWISAQMNRVRNGELYRYICDTKGAFFVQPAFYEAFFGLTVIESMTTCGLPTIAT
WSs1  GHIRWISAQMNRVRNGELYRYICDTKGAFFVQPAFYEAFFGLTVIEAMTCGLPTIAT
WSs2  GHIRWISAQMNRVRNGELYRYICDMKGAFFVQPAFYEAFFGLTVIEAMTCGLPTFAT
PSs   GQFRWISSQMNRVRNGELYRYIADTKGAFFVQPAFYEAFFGLTVVEAMTCGLPTFAT

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Fig. 2. A comparison of highly conserved amino acid sequences (corresponding to residue 643 to 697 of rice *RSs*2) of rice SS (*RSs*2, *RSs*3) isozymes with those of maize (*MSh*1), wheat (*WSs*1, *WSs*2) and potato (*PSs*) SS.

75

RSs1 gttacattgagatgttctatgctctgaaataccgcagcctggtaagttactctcgtgttcaacagcttgtaac
 RSs2 gctggccaacatctcaggctgagactctgaaagaggagagcattccagtttctttgctttgttccattctctgg
 RSs3 gacacgccgtacctggagatgctgtacgccctcaagtaccgcaagatggctaccaccgttccattggccattga

150

RSs1 ttcagtattattgcaactgaacatatctctgaattctgttgcattttctttgttgaatccaagctagctgagtc
 RSs2 tttcagagatgaaaagagaagagatatcttcaggatgctttgcattcctaggcactgctctttttccttaagaa
 RSs3 gggagaggcctccaccaaatgatctggccttaccgggtgaaaagaatgggcaatgggtgctccattgttgagtc

225

RSs1 ccattggctgtcgtatggagagagcacatccaagcaatggaggggaaaaatatgcatcttcagtaggagaagccgtc
 RSs2 agtaaggctcattgaatttgggagttttctagtttgatttagttgggggtgagttcagtggaatgtgggtgtgtgg
 RSs3 ctgatccaggggtgaagaaaaacagaaatcgaggaaacgaatgcattccatttagtttctaagggttagttgattt

300

RSs1 agctgcattcgaatttgataatcttctgtagttgtcatttggcatccatgtttgccatggatgtgtactatgtct
 RSs2 ttgggtgctctgtctcctcttgttatttgggatgttacctatgaataatagaaacggccttggtgccgtttctgcaa
 RSs3 cagggccagttcttgtgggggttttcaatggaagaaattgatgtaatgctctggccttttcatggatactatgaat

368

RSs1 aaggtttcagtacttttgcgagcttttgggcagtgcttgccctcaaataaacgccgggttcttggtgtta
 RSs2 taatatattgtagcacccttgtttcca
 RSs3 gaaataaatgaataacaagattctcta

Fig. 3. A comparison of 3' flanking sequences of three rice cDNA clones up to poly(A)⁺ tails.

rather than type 1 of maize SS (EMBL data accession number X59046). Besides, among several other cDNA clones we sequenced, two with poly(A)⁺ tails had coding sequences that showed high homology toward the type 2 gene but were distinct from each other. The deduced C-terminal sequences of the three rice SS isozymes are compared in Fig. 1 and their homologies with other known SS are compared in Table 1. From Table 1, it can be seen that the same type of SS from different plant sources share a homology higher than those among different types from the same plant species. It is also seen that type 3 rice SS is closer to type 2 than to type 1.

When the known full-length deduced amino acid sequences of SS from different plant sources are compared, it is very interesting to find that the highest divergence is found at the C- and N-termini, especially at the latter where some deletions must be allowed to establish homologies. Other parts of the SS molecules are highly conserved in general. Among them, a long stretch of 55 residues starting from residue 643 (numbering of RSs2 deduced amino acid sequence) (Fig. 2) seems to be so well conserved that even few substitutions found there are by amino acids of

similar nature only. Whether one of the active sites resides in this region awaits future studies.

The highest divergence of the three rice cDNA's are found in the 3' flanking sequence up to the poly(A)⁺ tail (Fig. 3). The sequences found in these regions could be very useful in developing specific probes for the three SS genes.

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