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Full Length Research Paper

Sequence analysis of cereal sucrose synthase genes and isolation of sorghum sucrose synthase gene fragment

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Sorghum (*Sorghum bicolor* (L.) Moench) is an important staple food for about 500 million people in semi arid regions of the world. Recently, sorghum has been identified as a main plant species for the comparative analysis of grass genomes and as a source of beneficial genes for agriculture. Recent studies have shown that there is conservation of gene order at the chromosome level in rice, sorghum and maize. Therefore, a high-resolution alignment between these genomes will be needed to utilize them constructively for sorghum gene discovery. Sorghum sucrose synthase gene fragment was amplified by primers designed at conserved exon position of cereal sucrose synthases. Sorghum sucrose synthase gene fragment I shared homology with other cereal sucrose synthase at the exon positions 6, 7, 8, 9 and 10. Sorghum sucrose synthase fragment II shared homology from exon 2 to 6.

Key words: Sorghum, sucrose synthase, multiple sequence alignment.

INTRODUCTION

Sorghum, a C_4 grass that diverged from maize just 15 million years ago, is the fifth most important cereal grown worldwide (Doggett, 1988). Recently, sorghum has been identified as a key plant species for the comparative analysis of grass genomes and as a source of beneficial genes for agriculture. Sorghum is relatively small genome (750 Mbp) (Arumuganathan and Earle, 1991)) and incremental divergence from maize and rice (Doebley et al., 1990), make it ideally suited to aid the discovery and analysis of grass genes through comparative genomics. Sucrose synthase genes have been isolated from many of plants, mostly from starch storing plants such as maize (Werr et al., 1985; Hung et al., 1994), rice (Wang et al., 1992; Yu et al., 1992), wheat (Marana et al., 1990), potato (Salanoubat and Belliard, 1987), pea (Barratt et al., 2001) as well as *Arabidopsis thaliana* (Chopra et al., 1992; Martin et al., 1993).

Sucrose synthase [UDP-D-glucose (UDPG): D-fructose 2-glycosyl transferase, EC 2.4.1.13, *SuS*] catalyzes a reaction of sucrose and UDP to form fructose and UDPG, the latter being a precursor of complex saccharide biosynthesis (Chourey and Nelson, 1976; Chourey et al., 1991; Carlson and Chourey, 1996). This study reports on multiple sequence alignment of sucrose synthase gene sequences from different cereals and isolation and sequencing of sucrose synthase gene fragment from sorghum using primers designed at their conserved exons.

MATERIALS AND METHODS

Multiple sequence alignment

Sucrose synthase gene sequences of various cereals like rice, maize, and barley were accessed from NCBI Genbank database and multiple sequence alignment was done using MegAlign programme of DNA star software.

Isolation of plant DNA

Two weeks old sorghum seedlings grown in ½ MS medium (Murashige and Skoog, 1962) were taken for total genomic DNA isolation. Sorghum (var. CSV 15) seeds were kept in 0.1% $HgCl_2$ for 10 min with intermittent shaking. After decanting 0.1% $HgCl_2$ solution, the sorghum seeds were thoroughly washed in sterile dis-

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Table 1. Nucleotide sequences of primers.

Primers	Sequence
GP1 F	5’ GC GTCGAC CCAAGAGCTTGGTTTGGAGAAGG 3’
GP1 R	5’ GC TCTAGA CTGTGAACTGGCATGAGAAGTGG 3’
GP2F	5’ CTCCTCTCATCCCAATG 3’
GP2 R	5’ CCCCTTCTCCAAACCAAG 3’

GP- Gene specific primer; R- Reverse primer; F- Forward primer.

tilled water two times and then placed in tissue culture bottle containing ½ MS medium. Sorghum seeds were kept at 28°C (BOD incubator) for germination. Sorghum genomic DNA was isolated by CTAB method (Dellaporta et al., 1983). The isolated genomic DNA was treated with RNase to make it RNA free. DNA quantitation was carried out by agarose gel analysis. 2 - 5 µl of sorghum genomic DNA was loaded in 0.7% agarose gel. Known amount of I Kb ladder was loaded as control in adjacent well. The quantity of DNA in the sample was estimated by comparison with the intensity of 3 kb band by eye judgment.

PCR

Primer sequences were designed at the conserved coding region to amplify a sucrose synthase gene fragment from sorghum (Table 1). Purification of amplified DNA from PCR reactions was performed using Qiaquick PCR purification kit (QIAGEN, Germany). pBluescript and UA cloning vector (QIAGEN PCR Cloning Kit) was used to clone the PCR amplified product

Transformation of E. coli cells

One vial of competent cells was taken out from -70°C and thawed by keeping on the ice. 2 ml of plasmid DNA or 15 µl of ligation mixture was added and kept on ice for 30 min. The cells were subjected to heat shock at 42ºC for 2 min, immediately plunged on ice for 5 min. To this 900 µl of Luria broth was added and kept at 37°C with shaking speed of 220 rpm for 1 h. The Cells were pelleted at 10,000 rpm for 1 min and resuspended in 100 µl of Luria broth, plated and incubated at 37°C for growth of transformed cells.

Cloning and sequencing

The recombinant clone was sequenced using T_7 and SP_6 reverse primers. Sequencing was carried out by Sanger dideoxy DNA sequencing method.

Results

Multiple sequence alignment

Sucrose synthase is found in all plant tissues and is found at high levels particularly in sink tissues. In monocotyledonous plants sucrose synthase is encoded by a small gene family. Sucrose synthase gene sequences of cereals such as rice, maize, and barley were taken from NCBI Genbank data base and multiple sequence alignment was carried out using MegAlign programmer of DNA star software. Genes Suc S_2, RSs_1 and SUS_2 are highly conserved with respect to their exon and intron position and number. All the three genes have 16 exons. All the gene sequences compared have conserved gene sequences at their coding part and not in the non-coding region. 13th exon of rice RSS_2 is split in two. 14th exon of RS_2 is sharing homology with 16th exon of maize SuS_2 and rice RSS_1. In case of rice RSs_3 and Ss_2 of *Hordeum vulgare* the 3rd exon position is replaced with a new exon. This new exon of RSS_3 of rice and Ss_2 of *H. vulgare* does not share homology with 3rd exons of RSS_1, RSS_2 of rice, $SucS_2$, SuS_1 of maize, SS_2 of Saccharum *officinarum*. 5th exon of RSS_3 of rice and Ss_2 of *H. Vulgare* is missing in both. Various cereal sucrose synthase gene sequences taken for MSA are summarized in the table with their gene name, accession number, exon numbers, exon position and region of homology (Table 2)

Primer design and PCR amplification

As the sucrose synthase gene sequence is highly conserved among cereals especially at the coding region, from multiple sequence alignment, highly conserved exon positions were chosen to design primers and using those to amplify sucrose synthase gene fragment form sorghum. Schematic diagram showing the exon position where the primers were designed and sorghum sucrose synthase gene fragments were cloned is shown in Figure 1. The forward primer GP1F 5’ GC GTC GAC CCA AGA GCT TGG TTT GGA GAA GG 3’ was designed based on the region of homology at the exon position 6 and the reverse primer GP1R 5’ GC TCT AGA CTG TGA ACT GGC ATG AGA AGT GG3’ was designed based on the region of homology at the exon position 10. The purified sorghum genomic DNA was used for amplification. Various annealing temperatures ranging from 53 – 63°C were tested for amplification of the specific sequence. PCR reaction was run for 25 cycles and the reaction was electrophoresed on the 0.8% agarose gel. Among various annealing temperature tested, amplification was obtained at 56°C, which showed a single band of expected size of about 1 kb. No nonspecific amplification was observed (Figure 2). The amplified product was eluted from gel cloned in *Sal I* and *Xba I* pBluescript. To confirm the size of the insert, plasmid isolated from the transformed colony was digested with same two restriction enzymes. The expected fragment of ~1kb was obtained.

Cloning of sorghum sucrose synthase gene fragment

Sorghum sucrose synthase gene fragment cloned in pBluescript was sequenced and the sequence was aligned with other cereal sucrose synthase gene sequences, which were already taken for multiple sequence

Table 2. Gene name, accession number, exon numbers, exon position and region of homology different cereal sucrose synthases

S/N	Plant	Gene name	Access-ion No.	Exon No.	Alignment position	Exon position	Region of homology
1.	Oryza sativa	RSs$_1$	X 64770	2	20-130	2882-2992	2899-2996
	Oryza sativa	RSs$_2$	X 59046	2	20-120	2632-2770	2689-2765
	Oryza sativa	RSs$_3$	L 03366	2	40-100	14-123	52-133
	Zea mays	SucS$_2$	X 02382	2	10-110	2208-2321	2227-2325
	Hordeum vulgare	Ss$_2$	Y 15802	2	10-110	1333-1443	1333-1443
	Oryza sativa	Sus$_2$	L 39940	2	10-110	4191-4285	4205-4290
2.	*Oryza sativa*	RSs$_1$	X 64770	3	870-1060	3404-3524	3398-3581
	Oryza sativa	RSs$_2$	X 59046	3	880-990	3497-3627	3511-3630
	Oryza sativa	RSs$_3$	L 03366	3^{Δ}	-	658-787	Missing
				4^{*}	880-990	884-1035	857-872
	Zea mays	SucS$_2$	X 02382	3	870-1000	2833-3953	2827-2955
	Hordeum vulgare	Ss$_2$	Y 15802	3^{Δ}	700-820	1856-1985	1880-2015
				4^{*}	950-990	2064-2215	2110-2150
	Oryza sativa	Sus$_2$	L 39940	3	850-990	4697-4817	4703-4831
3.	*Oryza sativa*	RSs$_1$	X 64770	4	1090-1240	3614-3765	3607-4831
	Oryza sativa	RSs$_2$	X 59046	4	1090-1240	3722-3823	3725-3826
	Oryza sativa	RSs$_3$	L 03366	5^{∇}	119-1311	1142-1204	1142-1202
	Zea mays	SucS$_2$	X 02382	4	1090-1240	3041-3192	3031-3189
	Hordeum vulgare	Ss$_2$	Y 15802	5^{∇}	1150-1230	2304-2496	2297-2385
	Oryza sativa	Sus$_2$	L 39940	4	1090-1230	4907-5058	4910-5059
4.	*Oryza sativa*	RSs$_1$	X 64770	5	1360-1560	3849-4041	3846-4049
	Oryza sativa	RSs$_2$	X 59046	5	1360-1460 1480-1540	3994-4186	3994-4074 4104-4173
	Oryza sativa	RSs$_3$	L 03366	-	-	Missing$^{\alpha}$	-
	Zea mays	SucS$_2$	X 02382	5	1366-1470	3271-3464	3271-3477
	Hordeum vulgare	Ss$_2$	Y 15802	-	-	Missing	-
	Oryza sativa	Sus$_2$	L 39940	5	1360-1560	5142-5334	5151-5352
5.	*Oryza sativa*	RSs$_1$	X 64770	$6^{!}$	1650-1680 1690-1830	4042-4190	4136-4166 4178-4315^{0}
	Oryza sativa	RSs$_2$	X 59046	$6^{!}$	1720-1730	4312-4430	4316-4433
	Oryza sativa	RSs$_3$	L 03366	$6^{!}$	1720-1800	1489-1607	1496-1586
	Zea mays	SucS$_2$	X 02382	$6^{!}$	1710-1840	3615-3733	3604-3739
	Hordeum vulgare	Ss$_2$	Y 15802	$6^{!}$	1720-1830	2834-2952	2839-2959
	Oryza sativa	Sus$_2$	L 39940	$6^{!}$	1720-1830	5474-5602	5498-568
6.	*Oryza sativa*	RSs$_1$	X 64770	7	1940-2160	4405-4621	4400-4630
	Oryza sativa	RSs$_2$	X 59046	7	1840-2160	4550-4766	4549-4766
	Oryza sativa	RSs$_3$	L 03366	7	1940-2040	1695-1911	1697-1914
	Zea mays	SucS$_2$	X 02382	7	1950-2170	3832-4048	3829-4053
	Hordeum vulgare	Ss$_2$	Y 15802	7	1950-2150	3039-3255	3046-3251
	Oryza sativa	Sus$_2$	L 39940	7	1960-2160	5698-5914	5714-5924
7.	*Oryza sativa*	RSs$_1$	X 64770	8	2310-2450	4704-4799	4698-4826
	Oryza sativa	RSs$_2$	X 59046	8	2320-2420	4851-4946	4849-4949
	Oryza sativa	RSs$_3$	L 03366	8	2320-2410	2022-2117	2024-2119
	Zea mays	SucS$_2$	X 02382	8	2320-2430	4125-4219	4125-4223
	Hordeum vulgare	Ss$_2$	Y 15802	8	2320-2400	3411-3768	3603-3769
	Oryza sativa	Sus$_2$	L 39940	8	2320-2400	5997-6092	6001-6091

Table 2. Contd**.**

8.	*Oryza sativa*	RSs$_1$	X 64770	9	2520-2700	4896-5069	4891-5071
	Oryza sativa	RSs$_2$	X 59046	9	2530-2710	5028-5201	5029-5204
	Oryza sativa	RSs$_3$	L 03366	9	2530-2670	2233-2406	2240-2404
	Zea mays	SucS$_2$	X 02382	9	2550-2710	4315-4488	4315-4491
	Hordeum vulgare	Ss$_2$	Y 15802	9	2530-2700	3595-3768	3603-3769
	Oryza sativa	Sus$_2$	L 39940	9	2560-2700	9189-6362	6222-6372
9.	*Oryza sativa*	RSs$_1$	X 64770	10	2810-2940	5154-5270	5155-5277
	Oryza sativa	RSs$_2$	X 59046	10	2800-2930	5289-5905	5281-5408
	Oryza sativa	RSs$_3$	L 03366	10	2810-2930	2514-2630	2496-2634
	Zea mays	SucS$_2$	X 02382	10	2840-2920	4572-4688	4598-4680
	Hordeum vulgare	Ss$_2$	Y 15802	10	2840-2910	3852-3968	3882-3952
	Oryza sativa	Sus$_2$	L 39940	10	2830-2930	6447-6563	6466-6576
10.	*Oryza sativa*	RSs$_1$	X 64770	11	3040-3210	5353-5519	5354-5526
	Oryza sativa	RSs$_2$	X 59046	11	3040-3190	5489-5655	5486-5656
	Oryza sativa	RSs$_3$	L 03366	11	3040-3200	2726-2892	2725-2892
	Zea mays	SucS$_2$	X 02382	11	3040-3210	4798-4964	4800-4963
	Hordeum vulgare	Ss$_2$	Y 15802	11	3070-3210	4054-4220	4078-4228
	Oryza sativa	Sus$_2$	L 39940	11	3040-3200	6696-6812	6649-6819
11.	*Oryza sativa*	RSs$_1$	X 64770	12	3240-3540	5066-5832	5591-5838
	Oryza sativa	RSs$_2$	X 59046	12	3310-3450	5760-5984	5752-5819
	Oryza sativa	RSs$_3$	L 03366	12	3310-3540	2971-3195	2974-3206
	Zea mays	SucS$_2$	X 02382	12	3310-3480	5045-5269	5039-5210
	Hordeum vulgare	Ss$_2$	Y 15802	12	3310-3540	4304-4528	4308-4533
	Oryza sativa	Sus$_2$	L 39940	12	3310-3535	6901-7125	6903-7113
12.	*Oryza sativa*	RSs$_1$	X 64770	13	4110-4130 4200-4230	6172-6490	6212-6242 6286-6316
	Oryza sativa	RSs$_2$	X 59046	$13^{\square}$	4210-4240 3640-3700	6071-6389	6295-6325 6077-6137
	Oryza sativa	RSs$_3$	L 03366	$13^{\square}$	3640-3700 3790-3840	3283-3601	3292-3352 3442-3475
	Zea mays	SucS$_2$	X 02382	13	4090-4140 4240-4320	5404-5722	5417-54740 5547-5637
	Hordeum vulgare	Ss$_2$	Y 15802	$13^{\square}$	3640-3930	4607-4927	4616-4916
	Oryza sativa	Sus$_2$	L 39940	13	3700-3720	7465-7783	7221-7251
13.	*Oryza sativa*	RSs$_1$	X 64770	$14^{\blacksquare}$	4700-4810	6621-6865	6649-6748
	Oryza sativa	RSs$_2$	X 59046	$14^{\blacksquare}$	4030-4050	4688-6732	6467-6497
	Oryza sativa	RSs$_3$	L 03366	14	4120-4780 4860-4920	3688-3932	3734-3794 3859-3919
	Zea mays	SucS$_2$	X 02382	14	4720-4920	5851-6095	5891-6078
	Hordeum vulgare	Ss$_2$	Y 15802	14	4720-4920	5021-5265	5062-5249
	Oryza sativa	Sus$_2$	L 39940	$14^{\blacksquare}$	4720-4920	7914-8158	7964-8151
14.	*Oryza sativa*	RSs$_1$	X 64770	15	5110-5120 5190-5260	6977-7115	6986-6993 7030-7080
	Oryza sativa	RSs$_2$	X 59046	$15^{\blacksquare}$	5380-5400	6827-9225	7814-7844
	Oryza sativa	RSs$_3$	L 03366	15	5190-5230	4027-4165	4083-4105
	Zea mays	SucS$_2$	X 02382	15	5280-5290	6258-6396	6250-6260
	Hordeum vulgare	Ss$_2$	Y 15802	15	5190-5210	5374-5580	5428-5463
	Oryza sativa	Sus$_2$	L 39940	$15^{\blacksquare}$	5170-5220	5270-8408	8322-8353
15.	*Oryza sativa*	RSs$_1$	X 64770	16	5370-5400 5490-5495	7213-7456	7143-7173 7224-7239

Table 2. Contd.

	Oryza sativa	RSs$_2$	X 59046	-	-	-	-
	Oryza sativa	RSs$_3$	L 03366	16	-	4262-4538	-
	Zea mays	SucS$_2$	X 02382	16	-	6516-6811	-
	Hordeum vulgare	Ss$_2$	Y 15802	-	-	-	-
	Oryza sativa	Sus$_2$	L 39940	16	-	8506-8553	-

∆= 3rd exon of RSs$_3$ (*Oryza sativa*) and SS$_2$ (*Horgeum vulgare*) share homology with each other but does not share a region of homology with 3rd exon of other sucrose synthase gene sequences compared; * = 4th exon of RSs$_3$ and SS$_2$ share region of homology with 3rd exon of other sucrose synthase gene sequences compared; ∇= 5th exon of RSs$_3$ and SS$_2$ share region of homology with 5th exon of other sucrose synthase gene sequences compared.; α= RSs$_3$ and SS$_2$ does not have an exon to share homology with 5th exon of other sucrose synthase gene sequences compared. ! = 6th exon is highly conserved and shares highest region of homology in all the sucrose synthase gene sequences. ☐ = 13th exon of RSs$_2$ shares similarity with 13th exon of RSs$_3$ and Ss$_2$ and not with the RSs$_1$, SucS$_2$ and SVS$_2$. ■ = 14th exon of RSs$_2$ shares similarity with 14th exon of RSs$_1$ and Sus$_2$ and not with the RSs$_3$, Ss$_2$ and SucS$_2$. 0 = 15th exon of RSs$_2$ shares region of homology only with Sus$_2$ and not with other sucrose synthase gene sequences compared.

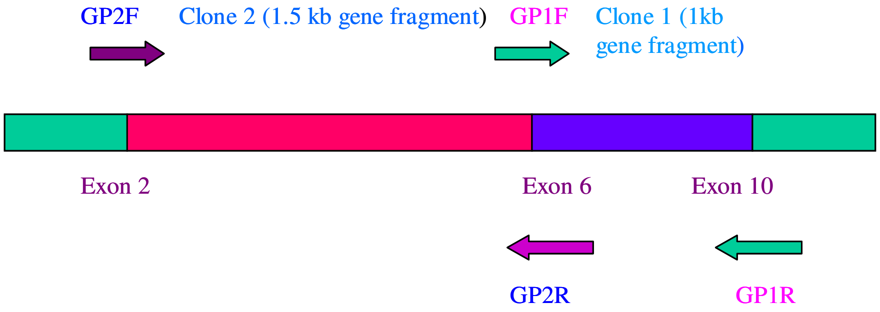

Figure 1. Schematic diagram showing the exon position where primers were designed and sorghum sucrose synthase gene fragments were cloned. GP- Gene specific primer; F - forward primer; R - reverse primer.

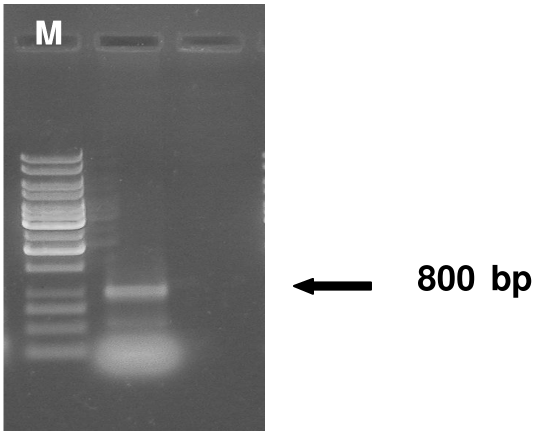

Figure 2. PCR Amplification of Sorghum sucrose synthase gene fragment using gene specific primers. Lane M 1 kb ladder. Lane 1 PCR Product of Sorghum sucrose synthase gene fragment I.

alignment. The cloned fragment shared homology in the region of exon 6, exon 7, exon 9 and exon 10 with Rss^1, Rss^2, and Rss^3 of rice, SUC^2, SUCI of maize, Ss$_2$ of *H. vulgare* and *S. officinanum*, so the PCR amplified and cloned fragment in pBluescript vector was confirmed as sucrose synthase gene fragment of sorghum (Figure 3).

To clone an upstream sequence of sucrose synthase gene, one more forward primer GP$_2$F was designed at the exon position 2 and reverse primer GP2F was designed at exon position 6. PCR amplification was done at various annealing temperatures ranging from 50 to 60°C using sorghum genomic as template. The electrophoresis of PCR products was done on 0.8% agarose gel. The sharp single band of expected size of about 1.5 kb was obtained at 55°C annealing temperature.

Thermostable polymerase enzyme will add extra adenine nucleotide at the end of the amplifying strand at each cycle. This property was utilized to clone a Taq amplified PCR product in TA cloning vector. The 1.5 kb PCR amplified product was cloned in pDrive TA cloning vector. Sequencing was done by Sanger dideoxy DNA sequencing method and the nucleotide sequence was given in Figure 4. The sequence of 1.5 kb gene fragment was compared with other cereal sucrose synthase. It shared homology at exon No. 2, 3, 4, 5, and 6 of RSS$_1$, RSS$_2$, RSS3 of rice, Suc 2, Suc1C of maize, SS2 of *H.*

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1 CAAGAGCTTG GTTTGGAGAA GGTTGGGGTG ACACTGCAAA GCGCCGTACT TGACACAC
61 CACTTGCTTC TTGACCTTCT TGAGGCCCCT GATCCTGCCA ACTTGGAGAA GTTCCTTG
121 ACTATACCAA TGATGTTNAA TGTTGTTATC CTGTCTCCTC ATGGCTACTT TGCCCAATC
181 AATGTGCTTG GATACCTTGA CACTGGTGGC CAGGTACAGA AGCTTAGTGA TTTTTTTT
241 AGACACTGAT TGTTTTTCTT TTAGCTATTA TAGCTTTTAG GTTTCTCATT TGCAATCATT
301 TTGCAGGTTG TGTACATTTT GGATCAAGTC CGTGCTTTGG AGAATGAGAT GCTTCTTA
361 ATTAAGCAGC AAGGCCTTGA CATCACCCCG AAGATCCTCA TTGTATGTTT CATGTTTG
421 ACCATGTTCG CCTTCTGAAC CCTTTTCGTA TGCTGGNTGA AGTCATGCAT TCTGTGCT
481 AGGATGTTGC CAGTGAAATA ATGTTAGAAA TGCAGGCCAA GCCTGACCTT ATCGTTG
541 ACTACAGTGA TGGCAACCTA GTCGCCACTC TGCTCGCACA CAAGTTGGGA GTTACTC
601 TCTGTTTGGC TGTACATGAA TAATTGAGTT TTTTTTTATA TAAAATTATT AAGTTCTCCA
661 AATGCCTAAT AGTTTTGTAC ATACTTGCAG TGTACCATTG CCCATGCCTT GGAGAAAA
721 AAATACCCCA ACTCGGACAT ATACTTGGAC AAATTTGACA GCCAATACCA CTTCTCAT

781 CAGTTCACAG TCTAGAGC
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Figure 3. Nucleotide sequence of Sorghum sucrose synthase gene fragment I Gp1F-Gp1R. Highlighted portions are exon sequences sharing homology with cereal sucrose synthases.

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1 CTCCTCTCAT CCCAATGAGC TGATTGCACT CTTCCTCCAG GTGGGCATAC CAAAATATG
61 AACTTGCATT TCATTTCCTG TACTGGAATT TGTTAATTTG GTATTCTCTT CATCCCAAAT
121 GTAAACACGA GCATATGCAA CTTCTTTCTT GGTTTCTTTT GTTAACACCA TCATGCATG
181 TAATTGCTAT TCATCATCGA CTCATTGATC ATATATAATG ATTTTATGAT CAGGAGATT
241 TTGATTGTAA AGCATAGTGT TGCTGCTCTT CAGTTTTTGA AGCCTTTTGG TTTGATTAG
301 ACAATTAGTT GATAAGACAG TATACTTTGT GGTACATCAT TTGGCAGATT GTTTGACTT
361 AGTTGGTACA GTGCCATTTA ATATTTACAT CCTTCAGATC TAAATAGGAT ATAAAATGT
421 CATCACAGCA GGGGAAAAGG TACATGATAT GAGATGTAAC ATCCATTTTA TTTGTGAA
481 TCACTTTTAC AGGTATGTTA ACCAGGGCAA GGGAATGCTT CAGCGCCATC AACTGCT
541 TGAGTTTGAT GCCCTGTTTG ATAGTGACAA GGAGAAGTAT GCGCCC?  (Gap of 250-30
TTCG AAGACTTTCT
601 TCGTGCTGCT CAGTAACACT GCTGAGATGC CTGCTTGAGT GATTGCGCCA GTCAACA
661 ACTTGTGCTG AGCTGATTTG AGCCTTCAAT GCTCGTTCCC TCGTCCTTCC ATGTCAAA
721 CCATGGGAAT GGAGTGCAAT TCTTAACCGA CACCTGTCTT CCAAGTTGTT ACAGGAC
781 GAGAGCCTGT ACCCATTGCT GAATTTCCTC AAAGCCCATA ACTACAAGGG CACGGTG
841 TTACAATTCA GAATCTTCCA AGCACATGCT TCACAATGGA TGATGACAAT ATTTATTTA
901 GAACTTTACA TAATCTGAAA ATGGATTAAA TGATGCCACC CAACTCCCTC ATTTGTAAG
961 CTTTTTTTTT TCTGTTACAG ACGATGATGT TGAATGACAG AATTCAGAGC CTCCGTGG
1021 TCCAGTCATC CCTTAGAAAG GCAGAAGAGT ATCTACTGAG TGTCCCTCAA GACACTC
1081 ACTCAGAGTT CAACCATAGG TGATTCATCA ATAAATTGTC CTTGCCATTT AACTTTGG
1141 GAACTAGCAA ATGTATTAAC TGCTTGTATG CCACCATGAT CTGCATTAGG TTCCAAG

1201 TTCCAAGAGC TTGGTTTGGA GAAGGGG
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Figure 4. Nucleotide sequence of Sorghum sucrose synthase gene fragment II Gp2F-Gp2R.

vulgare and *S. officinanum.* So the 1.5 kb fragment was confirmed as sorghum synthase gene fragment. Highlighted portions are exon sequences sharing homology with cereal sucrose synthases

DISCUSSION

Sucrose synthase is found in all plant tissues and is found at high levels particularly in sink tissues. In monocotyledonous plants sucrose synthase is encoded by a small gene family. Sucrose synthase gene sequences of cereals such as rice, maize, sugarcane and barley were taken from NCBI Genbank database and multiple sequence alignment was carried out using MegAlign programme of DNA star software. Genes Suc S_2, RSs_1 and SUS_2 are highly conserved with respect to their exon and intron position and number. All the three genes have 16 exons. All the gene sequences compared have conserved gene sequences at their coding part and not in the non-coding region. 13th exon of rice RSS_2 is split in two. 14th exon of RS_2 is sharing homology with 16th exon of maize SuS_2 and rice RSS_1. In case of rice RSs_3 and Ss_2 of *H. vulgare* the 3rd exon position is replaced with a new exon. This new exon of RSS_3 of rice and Ss_2 of *H. vulgare* does not share homology with 3rd exons of RSS_1, RSS_2 of rice, $SucS_2$, SuS_1 of maize, SS_2 of *S. officinarum*. 5th exon of RSS_3 of rice and Ss_2 of *H. vulgare* is missing in both. A long leader intron is characteristic of sucrose synthase genes (Werr et al., 1985, Wang et al., 1992; Yu et al., 1992). It is present in all the sucrose synthase genes isolated so far, except for ASuS I from Arabidopsis (Martin et al., 1993). The Cis elements present in the leader intron may be involved in gene regulation.

Sorghum sucrose synthase gene fragment cloned in pBluescript was sequenced and the sequence was aligned with other cereal sucrose synthase gene sequences, which were already taken for multiple sequence alignment. The cloned fragment shared homology in the region of exon 6, exon 7, exon 9 and exon 10 with Rss[1], Rss[2] and Rss[3] of rice, SUC[2], SUCI of maize, Ss_2 of *H. vulgare* and *S. officinanum*. Therefore the PCR amplified and cloned fragment in pBluescript vector was confirmed as sucrose synthase gene fragment of sorghum.

It was decided to amplify the gene portion between exon 2 and exon 6. Primers were designed at exon 2 and exon 6 and 1.5 kb sucrose synthase gene fragment II was PCR amplified and cloned in pDrive cloning vector. The nucleotide sequence, which shared sequence similarity at their exon positions 2 to 6 with other cereal sucrose synthases. The position and the length of sequence homology were highlighted in the Figure 4. Exonic sequences not encoding protein (exon 1 and 16) of all the sucrose synthases taken for comparison do not exhibit conservation as that of coding region of the gene.

High level sequence divergence of the introns relative to that of exons was observed. This indicates that selection pressure against mutation abolishing or reducing the function of the gene. It was observed that sequences at exon/intron boundaries are highly conserved. These findings suggest that mutations in such a manner do not alter their ability to be recognized as introns. A very small percentage of sequence variations in exonic sequences encoding protein observed is an indication of the signifycance of evolution.

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