

Analysis of Expressed Sequence Tags (ESTs) from *Agrostis* Species Obtained Using Sequence Related Amplified Polymorphism

Gizem Dinler · Hikmet Budak

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Abstract Bentgrass (*Agrostis* spp.), a genus of the Poaceae family, consists of more than 200 species and is mainly used in athletic fields and golf courses. Creeping bentgrass (*A. stolonifera* L.) is the most commonly used species in maintaining golf courses, followed by colonial bentgrass (*A. capillaris* L.) and velvet bentgrass (*A. canina* L.). The presence and nature of sequence related amplified polymorphism (SRAP) at the cDNA level were investigated. We isolated 80 unique cDNA fragment bands from these species using 56 SRAP primer combinations. Sequence analysis of cDNA clones and analysis of putative translation products revealed that some encoded amino acid sequences were similar to proteins involved in DNA synthesis, transcription, and signal transduction. The cytosolic glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene (GenBank accession no. EB812822) was also identified from velvet bentgrass, and the corresponding protein sequence is further analyzed due to its critical role in many cellular processes. The partial peptide sequence obtained was 112 amino acids long, presenting a high degree of homology to parts of the N-terminal and C-terminal regions of cytosolic phosphorylating GAPDH (GapC). The existence of common expressed sequence tags (ESTs) revealed by a minimum evolutionary dendrogram among the *Agrostis* ESTs indicated the usefulness of SRAP for comparative genome analysis of transcribed genes in the grass species.

Keywords Bentgrass species · Expressed sequence tag (EST) · Sequence related amplified polymorphism

G. Dinler · H. Budak (✉)

Faculty of Engineering and Natural Sciences, Biological Science and Bioengineering Program,
Sabanci University, Orhanli, Tuzla-Istanbul 34956, Turkey
e-mail: budak@sabanciuniv.edu

Introduction

Agrostis comprises more than 200 species of perennial cool-season turfgrasses belonging to the Poaceae family (Hitchcock 1971). Creeping bentgrass (*Agrostis stolonifera* L.) can be propagated sexually by seeds and is used for golf greens and fairways in temperate climates (Warnke 2003), whereas colonial (*A. capillaris* L.) and velvet (*A. canina* L.) bentgrasses are rarely used in golf courses (Brilman 2003; Rummele 2003). Two other *Agrostis* species are reported as being used as turfgrass in the United States, dryland (*A. castellana* Boiss. and Reut.) and redtop (*A. gigantea* Roth) (Chakraborty et al. 2005). The growth habit of creeping and velvet bentgrasses is stoloniferous and adapted to cool and humid regions, whereas colonial bentgrass has a bunch-type growth habit (Brilman 2003). Like other grass species, *Agrostis* species have different ploidy levels with different genome composition. Velvet bentgrass is a diploid, with $x = 7$ and $2n = 2x = 14$, having only bivalents in the first metaphase of meiosis with an A_1A_1 genome composition (Jones 1956; Nelson 1985). Creeping bentgrass is reported to be allotetraploid, with $2n = 4x = 28$ chromosomes with an $A_2A_2A_3A_3$ genome (Warnke 2003). Colonial bentgrass also has $2n = 4x = 28$ chromosomes but has a different genome composition ($A_1A_1A_2A_2$ or $A_2A_2A_2A_2$) (Rummele 2003).

Sequence related amplified polymorphism (SRAP) technology has been recognized as one of the most variable types of DNA sequences found in plants. This system has been employed for mapping and gene tagging in *Brassica* (Li and Quiros 2001). SRAP has also been employed to evaluate genetic diversity and phenetic relationships among a broad range of cool- and warm-season turfgrass species (Budak et al. 2004a), tomato (*Lycopersicon esculentum* L. Mill.) (Ruiz et al. 2005), and squash (*Cucurbita moschata*) (Ferriol et al. 2003), and genetic relationships among subpopulations of single species (Budak et al. 2004b, 2005a). It has proven to be more informative than AFLP (amplified fragment length polymorphism), RAPD (rapid amplified polymorphic DNA), ISSR (inter-simple sequence repeat), and SSR (simple sequence repeat) markers (Ferriol et al. 2003; Budak et al. 2005a, 2004c). SRAP is based on two-primer amplifications where the primers are 17 or 18 nucleotides long. Primers consist of a core sequence of 13 or 14 bases, where the 5'-most 10 or 11 bases are nonspecific, followed by the sequence CCGG in the forward primer and AATT in the reverse primer. The core sequence is followed by three selective nucleotides at the 3' end of each primer (Li and Quiros 2001).

The objective of this study was to investigate the presence and nature of SRAP at the cDNA level (not genomic DNA) in three bentgrass species: creeping bentgrass (*Agrostis stolonifera*), velvet bentgrass (*A. canina*), and colonial bentgrass (*A. capillaris*).

The study also includes characterization of a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene identified from velvet bentgrass, because of its critical role in many cellular processes, from both an enzymological and a molecular point of view. For instance, up-regulation of this GAPDH gene has been shown to increase tolerance under various stress conditions, such as in response to environmental stress in maize (Russell and Sachs 1989), *Arabidopsis thaliana* (Yang et al. 1993), and *Craterostigma plantagineum* (Velasco et al. 1994), and biotic stress in potatoes (Laxalt et al. 1996).

Materials and Methods

Plant Materials

Approximately 1,000 pure live seeds from each of 'Southshore' creeping bentgrass (*Agrostis stolonifera*), 'SR7200' velvet bentgrass (*A. canina*), and 'Heriot' colonial bentgrass (*A. capillaris*) species were planted in the greenhouse to insure that the resulting plant populations represented the diversity of each species. The seeds were planted in pots 15 cm in diameter and 15 cm deep, with a soil mixture of 350 g kg⁻¹ peat, 320 g kg⁻¹ vermiculite, 90 g kg⁻¹ soil, and 240 g kg⁻¹ sand. The greenhouse was maintained at 25±1°C with supplemental light supplied by metal halide lamps on a photoperiod of 15 h day/9 h night. The pots were saturated biweekly with a soluble fertilizer (21 N, 3.5 P, 15 K) at 200 mg l⁻¹ nitrogen (Budak et al. 2005b). Individual seedlings of each *Agrostis* species were used for cDNA amplification using 56 SRAP primer pair combinations.

cDNA Library Construction

Total RNA was isolated from 0.2 g fresh leaf and root tissues using an RNeasy Plant Mini Kit (Qiagen), and poly(A)⁺RNA was isolated from total RNA using the Oligotex mRNA Mini Kit. Results were visualized by electrophoresis in formaldehyde (2.2 M)-agarose (1.2%) gel in MOPS buffer (0.02 M MOPS, 2 mM CH₃COONa, 1 mM EDTA) at pH 7.0. Isolated RNA was then treated with DNaseI and precipitated before the determination of its concentration and purity spectrophotometrically. A λ-ZAP cDNA library was constructed with 5 µg of mRNA from leaf and root tissue following the manufacturer's instructions (Stratagene, La Jolla, CA).

Amplification, Cloning, and Sequencing of cDNA Using SRAP

Total RNA (2.5 µg) was converted to cDNA using an Omniscript reverse transcription kit (Qiagen) according to the manufacturer's instructions. First-strand cDNA was synthesized from the poly(A)⁺ RNA with Moloney Murine Leukemia Virus (MMLV) reverse transcriptase. In total, 56 SRAP oligonucleotide combinations (Table 1) were used for PCR amplification of cDNA sequences from three bentgrass species. Amplification of cDNA fragments was performed in 20 µl PCR reactions (Budak et al. 2005a, 2006). Each reaction mixture contained 2 µl first-strand cDNA, 2 µl 10 × PCR buffer without MgCl₂, 2.5 mM MgCl₂, 200 µM dNTP mix, 1 µM forward primer, 1 µM reverse primer, 1.25 U *Taq* DNA polymerase (Promega). Amplifications were carried out using an MJ Research PTC-100 thermocycler programmed for 32 cycles of 1 min at 94°C, 1 min at 50°C, 1 min at 72°C, followed by a final extension at 72°C for 5 min before cooling to 24°C. The PCR products obtained from first-strand cDNA synthesis were size-fractionated in 6% polyacrylamide (PAGE)/8 M urea gel. The gel containing 10 µl of amplification product was separated at 180 V for 4 h.

Table 1 SRAP primers used in this study

Forward Primers ^a		Reverse Primers ^a	
Me1	TGA GTC CAA ACC GGA TA	Em1	GAC TGC GTA CGA ATT AAT
Me2	TGA GTC CAA ACC GGA GC	Em2	GAC TGC GTA CGA ATT TGC
Me3	TGA GTC CAA ACC GGA AT	Em3	GAC TGC GTA CGA ATT GAC
Me4	TGA GTC CAA ACC GGA CC	Em4	GAC TGC GTA CGA ATT TGA
Me5	TGA GTC CAA ACC GGA AG	Em5	GAC TGC GTA CGA ATT AAC
Me6	TGA GTC CAA ACC GGA CA	Em6	GAC TGC GTA CGA ATT GCA
Me7	TGA GTC CAA ACC GGA CG	Em7	GAC TGC GTA CGA ATT CAA
Me8	TGA GTC CAA ACC GGA CT	Em8	GAC TGC GTA CGA ATT CAC
Me9	TGA GTC CAA ACC GGA GG	Em9	GAC TGC GTA CGA ATT CAG
Me10	TGA GTC CAA ACC GGA AA	Em10	GAC TGC GTA CGA ATT CAT
Me11	TGA GTC CAA ACC GGA AC	Em11	GAC TGC GTA CGA ATT CTA
Me12	TGA GTC CAA ACC GGA GA	Em12	GAC TGC GTA CGA ATT CTC
Me13	TGA GTC CAA ACC GGA AG	Em13	GAC TGC GTA CGA ATT CTG
Primer Combinations ^b			
Em6–Me1	Em8–Me3	Em6–Me2	Em8–Me4
Em6–Me3	Em8–Me5	Em6–Me4	Em8–Me6
Em6–Me5	Em8–Me7	Em6–Me6	Em8–Me8
Em6–Me7	Em8–Me9	Em6–Me8	Em8–Me10
Em6–Me9	Em8–Me11	Em6–Me10	Em8–Me12
Em6–Me11	Em8–Me13	Em6–Me12	Em10–Me1
Em6–Me13	Em10–Me2	Em7–Me1	Em10–Me3
Em7–Me2	Em10–Me5	Em7–Me3	Em10–Me9
Em7–Me4	Em10–Me10	Em7–Me5	Em10–Me11
Em7–Me6	Em10–Me12	Em7–Me7	Em11–Me4
Em7–Me8	Em11–Me5	Em7–Me9	Em11–Me7
Em7–Me10	Em11–Me9	Em7–Me11	Em11–Me10
Em8–Me1	Em11–Me12	Em8–Me2	Em11–Me13
Em1–Me1	Em1–Me3	Em1–Me5	Em1–Me12

^a Li and Quiros, 2001

^b Budak et al. 2005a

Amplified fragments were excised from gels and extraction was performed using the QiaexII Gel Extraction Kit (Qiagen). DNA was inserted into a PGEM-T Easy Vector System I (Promega, Madison, WI) and propagated in *E. coli* DH5 (Life Technology) grown cells in Luria Bertani medium containing 100 µg/ml ampicillin. Three clones were selected for sequence analysis.

Sequence Analysis

The sequences were first entered in VecScreen <http://www.ncbi.nlm.nih.gov/VecScreen/VecScreen.html>, which aligns the entered sequences with common vector sequences. Using this tool, the vector sequences were identified and removed. The

Blast algorithm (Altschul et al. 1997) was used to analyze the DNA sequences (<http://www.ncbi.nlm.nih.gov>). The discontinuous MegaBlast tool (<http://www.ncbi.nlm.nih.gov>) was used because the regular MegaBlast tool gave only exact or highly similar matches, causing some similar sequences to be ignored. The “nr” database was used (all GenBank + RefSeq Nucleotides + EMBL + DDBJ + PDB sequences). The sequences obtained were then submitted to dbEST, GenBank, under the accession numbers EB812786–EB812823. Sequence analysis of the cDNA fragment EB812822 was further translated to protein sequence using the Translate tool at the Expasy Web site (<http://www.expasy.org/tools/dna.html>). Sequence alignment of the corresponding protein sequence of the EB812822 EST fragment was done by ClustalW (Vector NTI version 9.0) against various plant and mammalian (wheat, maize, barley, corn, banana, lobster, halophyte, carnation) cytosolic phosphorylating GAPDH sequences.

Expressed Sequence Tag (EST) Analysis

Minimum evolutionary analysis was examined with a Dayhoff model tree constructed using the PAM matrix (Mega program, version 3.1). The PAM distance matrix primarily corrects for multiple substitutions based on the model of amino acid substitution outlined as substitution-rate matrices and uses the PAM 001 matrix (Schwarz and Dayhoff 1979). Using a substitution-rate matrix (Q), the matrix F , which consists of the observed proportions of amino acid pairs between a pair of sequences with their divergence time t , is given by the following equation:

$$F(t) = Ae^{2tQ}$$

where A is the diagonal matrix of the equilibrium amino acid frequencies for Q . From the equation, the evolutionary distance $d = 2tQ$ was computed by a maximum-likelihood method. The eigen values for the PAM matrix were obtained from the program source code of Phylip version 3.6 (Felsenstein 1993).

Results and Discussion

Identification and Characterization of ESTs Using SRAP

A total of 56 SRAP oligonucleotide combinations (Table 1) were used for cDNA amplifications of three bentgrass species: creeping bentgrass (*Agrostis stolonifera*), velvet bentgrass (*A. canina*), and colonial bentgrass (*A. capillaris*). The PCR products generated 80 fragment bands in the size range 250–950 bp. Fragment bands smaller than 100 bp were not visible on the gel. A total of 38 cDNA fragment bands that appear to be unique within each species (19 from creeping, 10 from colonial, and 9 from velvet bentgrass species) were excised from the gel. Three cDNA clones per fragment were sequenced and analyzed further. Some SRAP markers worked in the creeping bentgrass genome but did not amplify cDNA in colonial and velvet bentgrasses. This response might be because velvet bentgrass does not contain the amplification site when compared to creeping bentgrass. This degree of site loss likely reflects genomic divergence in *Agrostis* species.

The SRAP primer combinations used targeted and amplified ORFs. The possible ORFs were determined by the ORF finder program at <http://www.ncbi.nlm.nih.gov/gorf/gorf.html> (Table 2). A GenBank search with the BlastX algorithm (Altschul et al. 1997) was performed to compare the sequences of ESTs obtained to the nr databases. A homology search revealed similarity in 68% (26/38) of ESTs to functionally diverse proteins cloned from other plants, bacteria, virus species, and zebrafish species. Identified ESTs revealed significant similarity to proteins involved in DNA synthesis, transcription, and transposon elements such as ribonucleotide reductase, putative reverse transcriptase, and retrotransposon protein, and proteins involved in structures like Matrilin 2 (MATN2, cell wall-associated kinases); extensin-like proteins; and metabolism-related enzymes such as phosphoglucosyltransferase, GAPDH, and catalase. Particularly, the corresponding protein of the EST sequence, EB812822, reveals clear sequence homology (93%) to the ubiquitous and highly conserved cytosolic phosphorylating GAPDH (GapC) enzyme of *Triticum aestivum* (EF592180). Hence GAPDH and its deduced amino acid sequence were further analyzed. Sequence alignments of representative homologs from plants and eukaryotes are shown in Fig. 1.

Sequence Analysis of Cytosolic GAPDH

The partial peptide sequence obtained is 112 amino acids long (complementary amino acids span from 227 to 330). One monomeric unit of the GAPDH comprises two conserved functional domains, an N-terminal NAD-binding domain (residues 1–147 and 312–330) and a C-terminal catalytic domain (residues 148–311) (Yun et al. 2000; Branlant and Branlant 1985) (in the text all the residue numberings are given according to the lobster GAPDH sequence unless otherwise noted) (Fig. 2). Some of the known residues that are highly conserved in the GAPDH sequences are present in velvet bentgrass cDNA. The two critical amino acids for the catalytic mechanism of GAPDH, Cys 149 and His 176, were not sequenced in the cDNA fragment of velvet bentgrass. Arg 231 is conserved in the obtained EST, which implies the presence of the putative second anion recognition site. Also, Tyr 311 and Ser 238, which are identified as conserved residues in the cDNA fragment obtained, are the two hydrogen-bonded residues to His 176 in the two dimeric subunits of the homotetramer (Moras et al. 1975). One of the interfacial hydrogen-bond forming residues, Glu 276, in the catalytic domain (Skarzynski et al. 1987) is also conserved in the EST sequence obtained (Fig. 2). The active site loop, 220s loop, whose sequence is highly conserved among GAPDH, is partially obtained in the velvet bentgrass (residues Gly 227 to Phe 230). As observed for the available GAPDH structures, this loop acts like a latch and closes toward the catalytic Cys 149 and His 176 residues to constrain the active site by moving the inorganic phosphate-binding site away from the active region (Kim and Hol 1998).

EST Analysis

A minimum evolution dendrogram was constructed among the sequences (Fig. 3) in order to further analyze the EST obtained. The cophenetic correlation between

Table 2 ESTs and homologous sequences obtained by BlastX search at the NCBI network server

GenBank acc. no.	Homology ^a	Probability	ORF	
			Frame	Begin End
EB812786	NSS		+3	3 115
EB812787	Pkcs-h-prov protein-like [<i>Oryza sativa</i> (japonica cultivar-group)]; Protein kinase C substrate 80K-H [<i>Xenopus tropicalis</i>]	0.61	+2	62 205
EB812788	Extensin-like protein [<i>Cucumis sativus</i>]	8.8	+3	3 188
EB812789	Hypothetical protein Dace_0745 [<i>Desulfuromonas acetoxidans</i> DSM 684]	1.1	-3	1 201
EB812790	Putative reverse transcriptase [<i>Cicer arietinum</i>]; Catalase (EC 1.1.1.6) CAT-2-maize (fragment)	3e-12 3e-8	-2	358 666
EB812791	Acyl-activating enzyme 11, putative, expressed [<i>Oryza sativa</i> (japonica cultivar-group)]	0.057	-1	29 144
EB812792	NSS		-3	1 122
EB812793	NSS		-3	1 134
EB812794	Transposon protein, putative, CACTA, En/Spm sub-class [<i>Oryza sativa</i> (japonica cultivar-group)]; Transposase [<i>Zea mays</i>]	0.015 0.013	+1	1 143
EB812795	Retrotransposon protein, putative, unclassified [<i>Oryza sativa</i> (japonica cultivar-group)]; Retrotransposon protein, putative, unclassified [<i>Oryza sativa</i> (japonica cultivar-group)]	0.012 1.4	+3	3 131
EB812796	NSS		+1	217 447
EB812797	Putative wall-associated kinase [<i>Oryza sativa</i> (japonica cultivar-group)]	0.001		
EB812798	Wall-associated kinase 3 [<i>Triticum aestivum</i>] Cation/multidrug efflux pump, ActB/ActD/AcrFfamily, RND superfamily [<i>Burkholderia xenovorans</i> LB400]	0.18 6.7	-3 -2	232 363 226 393
EB812799	Hypothetical protein, conserved [<i>Trypanosoma brucei</i>]	0.21	-1	3 134
EB812800	Acetyl-CoA synthetase [<i>Syntrhopus aciditrophicus</i> SB]; Acetyl-coenzyme A synthetase [<i>Methylococcus capsulatus</i> str. Bath]	1.1 1.8	-1 -2	3 173 173 334

Table 2 continued

GenBank acc. no.	Homology ^a	Probability	ORF		
			Frame	Begin	End
EB812801	Homeodomain protein GhHOX2 [<i>Gossypium hirsutum</i>];	4			
EB812802	Homeodomain protein BNLGH6863 [<i>Gossypium hirsutum</i>]; GATA-binding protein 1 [<i>Danio rerio</i>];	5.2 6.9	+2	308	439
EB812803	Putative polyprotein [<i>Oryza sativa</i> (japonica cultivar-group)]	9	-2	1	231
EB812804	NSS Similar to dishevelled 1 isoform a, partial [<i>Danio rerio</i>]; Similar to Segment polarity protein dishevelled homolog DVL-1 (Dishevelled-1) (DSH homolog 1) [<i>Danio rerio</i>]	8.8 8.8	-2 +1	304	486 129
EB812805	NSS		+2	59	181
EB812806	NSS		-1	26	156
EB812807	Unnamed protein product [<i>Tetraodon nigroviridis</i>]; Unnamed protein product [<i>Tetraodon nigroviridis</i>]	4 8.9	+3 +1	69	287 120
EB812808	NSS	0.17			
EB812809	Similar to MATN2 [<i>Pan troglodytes</i>]; Hypothetical protein [<i>Gordonia westfalica</i>]	1.1	+2	2	346
EB812810	NSS		-3	1	157
EB812811	Expressed protein [<i>Oryza sativa</i> (japonica cultivar-group)]; Hypothetical protein [<i>Oryza sativa</i> (japonica cultivar-group)]	0.03 0.096	-3	1	201
EB812812	SJCHGC09350 protein [<i>Schistosoma japonicum</i>]; Phosphoglucosyltransferase [<i>Chlamydia trachomatis</i> A/HAR-13]	9 9	+1	1	189
EB812813	Putative gag-pol polyprotein [<i>Oryza sativa</i> (japonica cultivar-group)]; Gag-pol polyprotein [<i>Zea mays</i>]	6e-26 1e-22	+3	156	374
EB812814	NSS		-3	1	99

Table 2 continued

GenBank acc. no.	Homology ^a	Probability	ORF	
			Frame	Begin End
EB812815	Hypothetical protein TA08890 [<i>Theileria annulata</i> strain Ankara];	1.3		
EB812816	Glutamate receptor family (ampa) protein 6, isoform a [<i>Caenorhabditis elegans</i>]; Diguanylate cyclase (GGDEF domain) [<i>Polaromonas</i> sp. JS6661];	3	-3	75 320
EB812817	Hypothetical protein CBG01104 [<i>Caenorhabditis briggsae</i>]	5.2	+2	206 400
EB812818	COG0209: Ribonucleotide reductase, alpha subunit [<i>Mycobacterium tuberculosis</i> C]	3.1	-3	1 102
EB812819	NSS	9.4	+1	1 144
EB812820	Rhs family protein [<i>Xanthomonas campestris</i> pv. vesicatoria str.]; Putative tetrapeptide repeat (TPR)-containing protein [<i>Oryza sativa</i> (japonica cultivar-group)];	4e-40	-2	99 302
EB812821	Hypothetical protein [<i>Triticum aestivum</i>] Envelope glycoprotein [Seoul virus K24-e7];	3e-29	-2	1 270
EB812822	G1/G2 glycoprotein [Seoul virus] Cytosolic glyceraldehyde-3-phosphate dehydrogenase [<i>Triticum aestivum</i>]; Glyceraldehyde-3-phosphate dehydrogenase [<i>Zea mays</i>]	2.4	-2	1 240
EB812823	NSS	6e-50	+2	14 331
		6e-49	-1	209 609

^a NSS, no significant similarity

	230	240	250	260	270	280
Halophyte	LTGMSFRVPTVDVSVV	DLTVRLEKEATY	EQIKAAIK EESE	SGMK GILGY	EDDV VSTDFV	
Carnation	LTGMSFRVPTVDVSVV	DLTVRIEKPATY	EQV KAAIKEESE	SGK LKGILGY	EDDV VSTDFV	
Banana	LTGMSFRVPTVDVSVV	DLTVRLEKAATY	DEIKAAIK EESE	SGK LKGILGY	EEDL VSTDFV	
Maize	LTGMSFRVPTVDVSVV	DLTVRLEKSATY	DEIKAAV KAAE	AGS LKGILGY	EEDL VSTDFQ	
Wheat	LTGMAFRVPTVDVSVV	DLTVRLAKPATY	DQIKAAIK EESE	SGN LKGILGY	DEDL VSTDFQ	
Barley	LTGMAFRVPTVDVSVV	DLTVRLAKPATY	EQIKAAIK EESE	SGN LKGILGY	DEDL VSTDFQ	
V. Bentgrass	SQGM	AFRVPTVDVSVV	DLTVRLEKSATY	DEIKAAIK AESE	GN LKGILGY	EEDL VSTDFQ
Lobster	LTGMAFRVPTPDVSVV	DLTVRLGK	ECSYDDIKAA MKTASE	GP LQ	CF LG	YIEDDV VSSDFI
	::***	*****::*	*.:*::**::*	::**	::*::**::*	::*::**::*
	290	300	310	320	330	
Halophyte	GDNRSSIFDAKAGI	QLNGNFVKLV	SWYDNEWGY	SSRV VDLIR	HMSK TTSKLDS	
Carnation	GDNRSSIFDAKAGI	ALNDNF	IKLVSWYDNEWGY	STRV VDL	IAH IKHTCQ----	
Banana	GDNRSSIFDAKAGI	ALNPNFAKL	VSWYDNEWGY	SSRV IDL	IRHM -----	
Maize	GDNRSSIFDAKAGI	ALNGNFVKLV	SWYDNEWGY	STRV VDLIR	HM NSTN----	
Wheat	GDNRSSIFDAKAGI	ALNDHFVKLV	SWYDNEWGY	STRV VDLIR	HM STK-----	
Barley	GDNRSSIFDAKAGI	ALNDNFVKLV	SWYDNEWGY	STRV VDLIR	H MSTK-----	
V. Bentgrass	GDNRSSIFDAKAGI	ALNDKFVKLV	SWYDNEWGY	SSRV VDLIR	H MSTNS	SGLT
Lobster	GDNRSSIFDAKAGI	QLSKTFVKV	VSWYDNE	FGYS Q	RV IDL	LKHM QKVDSA---
	**	*****	*	*	::*****	::**

Fig. 1 Sequence alignment of velvet bentgrass GAPDH and other phosphorylating plant and eukaryotic GAPDH amino acid sequences obtained using ClustalW program. Asterisks (*) indicate fully conserved residues. Bold letters in positions marked by a colon (:) indicate strong conservation among the species. Gray letters in positions marked by a dot (.) indicate weak conservation among the species. There is no consensus among the species for other amino acids

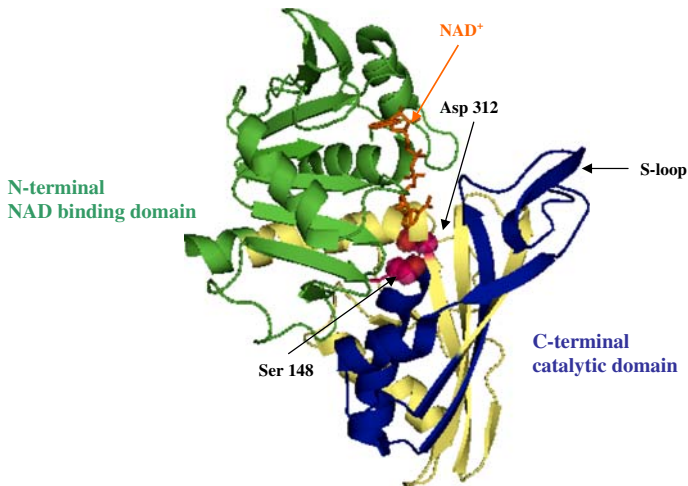


Fig. 2 Crystal structure of the lobster GAPDH monomer (Protein Data Bank code 1GPD). Residues represented as spheres, Ser 148 and Asp 312, are the linkage residues between the two domains of GAPDH, the N-terminal NAD-binding domain (green) and the C-terminal catalytic domain (blue). The yellow region of the ribbon diagram corresponds to the homologous parts of the GAPDH EST sequence obtained for velvet bentgrass. NAD^+ is shown as a stick model in orange. The S-loop is shown with a black arrow. The figure was produced using the program Pymol (DeLano 2002)

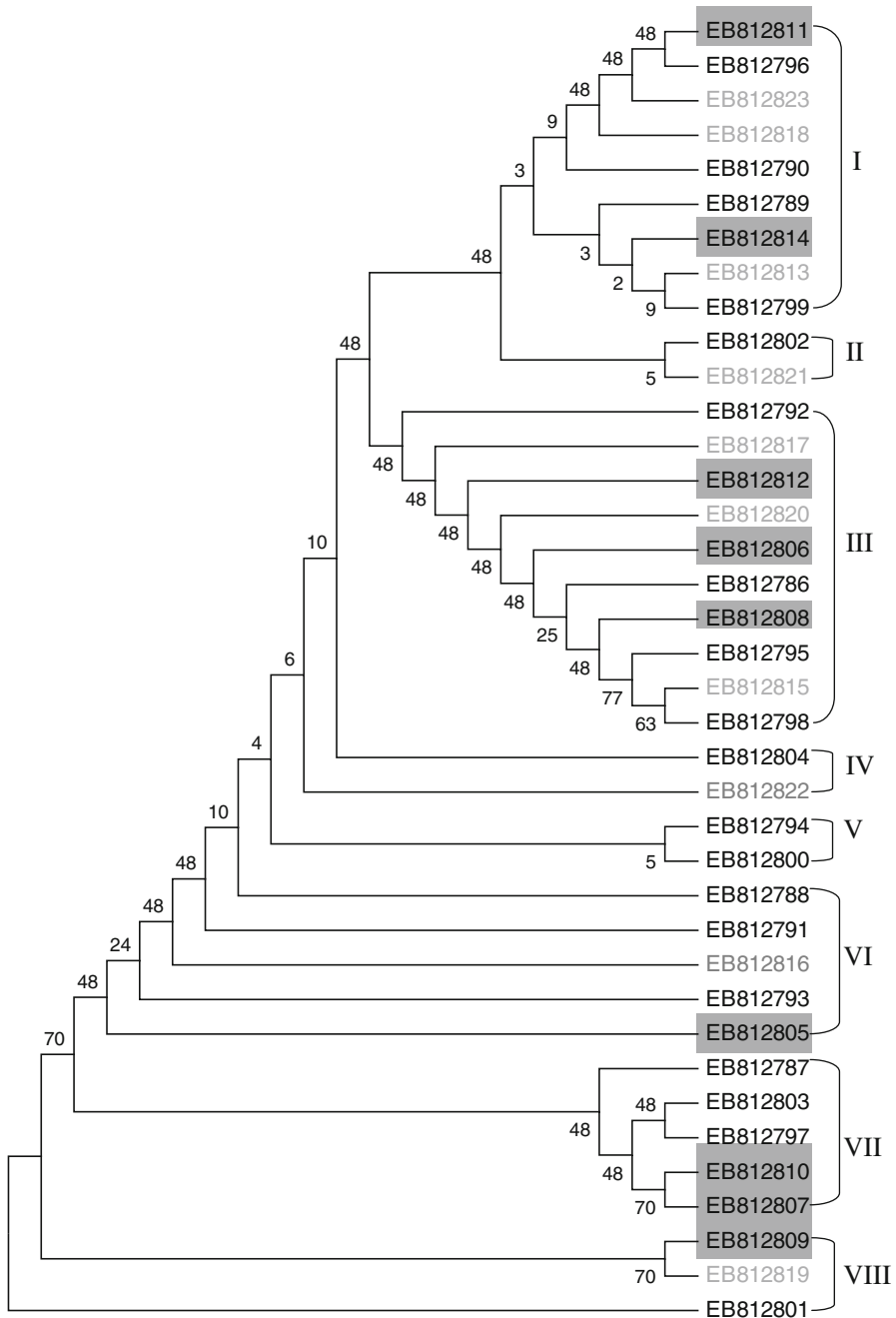


Fig. 3 Minimum evolution tree comparing translated EST sequences obtained from *Agrostis* species. Numbers at the nodes are the bootstrap percentages based on 500 replications. GenBank accession numbers EB812786–EB812804 are for creeping bentgrass (black type), EB812805–EB812814 for colonial bentgrass (gray screen), and EB812815–EB812823 for velvet bentgrass (gray type)

ultrametric similarities of the tree matrix was $r = 0.93$, suggesting the cluster analysis performed for this data strongly represents the similarity matrix. Eight clustering groups were found and are depicted in Fig. 3. The first group consists of 9 ESTs, including colonial, creeping, and velvet bentgrasses. There are 10 ESTs in the third group. Groups II and IV contain creeping and velvet bentgrass ESTs, but group V has only creeping bentgrass ESTs. Colonial bentgrass is known to be more related to creeping bentgrass (Macbryde 2005); however, SRAP analysis revealed more common ESTs among creeping and velvet bentgrass species. Group VII consisted of five ESTs from creeping and colonial bentgrasses, which might be an example of a gene from a common ancestor that later diverged during speciation. The two ESTs, EB812811 from colonial bentgrass and EB812801 from creeping bentgrass, were found to span the extremes of the dendrogram, indicating that these ESTs have the highest divergence. These results indicated that the SRAP primer combinations, targeted at the transcribed genes of three bentgrass species, revealed the existence of common ESTs. Different clustering groups might indicate that these cDNA sequences isolated from three C3 *Agrostis* species appear to be part of a multigene family.

Conclusion

Cloning and sequencing of a set of cDNA to visualize transcript polymorphism are reported using SRAP technology in three bentgrass species. There are no reports on the use of SRAP for revealing transcribed bentgrass species genes or any other turfgrass species. The ESTs identified in this study could potentially be used in turfgrass breeding and genetics programs as functional markers. Integration of these ESTs to the existing linkage map of turfgrass species provides high-density coverage in selected genomic regions. Minimum evolutionary tree clustering indicated that ESTs obtained using SRAP could be used for comparative genomics analysis of transcribed genes among the grass species.

Because GAPDH plays a critical role in many cellular processes, from both an enzymological and a molecular point of view, this enzyme appears to be an attractive candidate for use as the target enzyme for *Agrostis* species. GAPDH can be used as a molecular biomarker because of its ubiquitous distribution and slow rate of evolution (Fothergill-Gilmore and Michels 1993). Also, up-regulation of plant GAPDH under stress conditions implies that enhanced rates of glycolysis are one of the immediate responses to stress (Russell and Sachs 1989; Yang et al. 1993; Velasco et al. 1994; Laxalt et al. 1996). The identification, molecular characterization, and understanding of the processes by which plant GAPDH activities limit cellular damage may represent a critical strategy for surviving stress in plants; therefore, they could have a potential to improve stress tolerance in these species.

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References

- Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped Blast and PSI-Blast: a new generation of protein database search programs. *Nucleic Acids Res* 25:3389–3402
- Branlant G, Branlant C (1985) Nucleotide sequence of the *Escherichia coli* gap gene. Different evolutionary behavior of the NAD⁺-binding domain and of the catalytic domain of glyceraldehyde-3-phosphate dehydrogenase. *Eur J Biochem* 150:61–66
- Brilman L (2003) Velvet bentgrass. In: Casler M, Duncan RR (eds) Turfgrass biology, genetics and breeding. Wiley, Hoboken, NJ, pp 201–205
- Budak H, Shearman RC, Gaussoin RE, Dweikat I (2004a) Application of sequence-related amplified polymorphism (SRAP) markers for characterization of turfgrass species. *HortScience* 39:955–958
- Budak H, Shearman RC, Parmaksiz I, Gaussoin RE, Riordan TP, Dweikat I (2004b) Molecular characterization of buffalograss germplasm using sequence related amplified polymorphism markers. *Theor Appl Genet* 108:328–334
- Budak H, Shearman RC, Parmaksiz I, Dweikat I (2004c) Comparative analysis of seeded and vegetative biotype buffalograsses based on phylogenetic relationship using ISSRs, SSRs, RAPDs, SRAPs. *Theor Appl Genet* 109:280–288
- Budak H, Shearman RC, Gulsen O, Dweikat I (2005a) Understanding ploidy complex and geographic origin of *Buchloe dactyloides* genome using cytoplasmic and nuclear marker systems. *Theor Appl Genet* 111:1545–1552
- Budak H, Shearman RC, Dweikat I (2005b) Evolution of *Buchloe dactyloides* based on cloning and sequencing of matK, rbcL, and cob genes from plastid and mitochondrial genomes. *Genome* 48:411–416
- Budak H, Su S, Ergen N (2006) Revealing constitutively-expressed resistance genes in *Agrostis* species using PCR-based motif directed RNA fingerprinting. *Genet Res* 88:165–175
- Chakraborty N, Bae J, Warnke S, Chang T, Jung G (2005) Linkage map construction in allotetraploid creeping bentgrass (*Agrostis stolonifera* L.). *Theor Appl Genet* 111:795–803
- DeLano WL (2002) The PyMOL molecular graphics system. *DeLano Scientific*, San Carlos, CA, USA. Available from: <http://www.pymol.org>
- Felsenstein J (1993) Phylip—phylogeny inference package (Version 3.5). University of Washington, Seattle
- Ferriol M, Pico B, Nuez F (2003) Genetic diversity of a germplasm collection of *Cucurbita pepo* using SRAP and AFLP markers. *Theor Appl Genet* 107:271–282
- Fothergill-Gilmore LA, Michels PA (1993) Evolution of glycolysis. *Prog Biophys Mol Biol* 59(2):105–235
- Hitchcock AS (1971) Manual of the grasses of the United States, 2nd edn. Dover Publications, New York
- Jones K (1956) Species differentiation in *Agrostis*. I. Cytological relationships in *Agrostis canina*. *J Genet* 54:370–376
- Kim H, Hol WG (1998) Crystal structure of *Leishmania mexicana* glycosomal glyceraldehyde-3-phosphate dehydrogenase in a new crystal form confirms the putative physiological active site structure. *J Mol Biol* 278:5–11
- Laxalt AM, Cassia RO, Sanllorenti PM, Madrid EA, Andreu AB, Daleo GR, Conde RD, Lamattina L (1996) Accumulation of cytosolic glyceraldehyde-3-phosphate dehydrogenase RNA under biological stress conditions and elicitor treatments in potato. *Plant Mol Biol* 30:961–972
- Li G, Quiros CF (2001) Sequence-related amplified polymorphism (SRAP) a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in Brassica. *Theor Appl Genet* 103:455–461
- Macbride B (2005) White paper: perspective on creeping bentgrass, *Agrostis stolonifera* L. Available from: http://www.aphis.usda.gov/about_aphis/creeping_bentgrass.shtml
- Moras D, Olsen KW, Sabesan MN, Buehner M, Ford GC, Rossman MG (1975) Studies of asymmetry in the three-dimensional structure of lobster D-glyceraldehyde-3-phosphate dehydrogenase. *J Biol Chem* 250:9137–9162
- Nelson EK (1985) Breeding and selection for rhizomatous colonial bentgrasses, *Agrostis tenuis* Sibth. PhD. thesis, Pennsylvania State University, University Park, PA
- Ruiz JJ, Garcia-Martinez S, Pico B, Gao MQ, Quiros CF (2005) Genetic variability and relationship of closely related Spanish traditional cultivars of tomato as detected by SRAP and SSR markers. *J Am Soc for Hortic Sci* 130:88–94

- Rummele BA (2003) Colonial bentgrass. In: Casler M, Duncan RR (eds) Turfgrass biology, genetics and breeding. Wiley, Hoboken, NJ, pp 187–200
- Russell DA, Sachs MM (1989) Differential expression and sequence analysis of the maize glyceraldehyde-3-phosphate dehydrogenase gene family. *Plant Cell* 1:793–803
- Schwarz R, Dayhoff M (1979) Matrices for detecting distant relationships. In: Dayhoff M (ed) Atlas of protein sequences. National Biomedical Research Foundation, Washington, DC, pp 353–358
- Skarzynski T, Moody PCE, Wonacott AJ (1987) Structure of the holo-glyceraldehyde-3-phosphate dehydrogenase from *Bacillus stearothermophilus* at 1.8 Å of resolution. *J Mol Biol* 193:171–187
- Velasco R, Salamini F, Bartels D (1994) Dehydration and ABA increase mRNA levels and enzyme activity of cytosolic GAPDH in the resurrection plant *Craterostigma plantagineum*. *Plant Mol Biol* 26:541–546
- Warnke SE (2003) Creeping bentgrass (*Agrostis stolonifera* L.). In: Casler M, Duncan RR (eds) Turfgrass biology, genetics and breeding. Wiley, Hoboken, NJ, pp 75–185
- Yang Y, Kwon HB, Peng H-P, Shih M-C (1993) Stress responses and metabolic regulation of glyceraldehyde-3-phosphate dehydrogenase genes in Arabidopsis. *Plant Physiol* 101:209–216
- Yun M, Park CG, Kim JY, Park HW (2000) Structural analysis of glyceraldehyde-3-phosphate dehydrogenase from *Escherichia coli*: direct evidence of substrate binding and cofactor-induced conformational changes. *Biochemistry* 39:10702–10710