ABSTRACT

Seagrass meadows are important primary producers and habitats in estuaries and near-shore marine environments, but many populations are in decline due to anthropogenic influences. Measurements of biomass are commonly used to gauge the physiological status of seagrass meadows, but these are "lagging indicators" of the underlying causal event(s) and have not fully answered questions about why, despite attempts to correlate with environmental conditions. The goal is to develop a method for transcriptomic measurements to compare relative long-term stress response levels between impacted and nonimpacted seagrasses. Given the lack of genomic information for seagrasses in the western Gulf of Mexico, it was first necessary to obtain seagrass genomic sequences based on knowledge of model systems. Control (*Act1, Gapdh*) and stress genes (*Apx1*, non-symbiotic *Hb*1, and *Pal1*) were first identified by literature search using the rice (*Oryza sativa*) genome as a model. Multiple alignments were performed to identify conserved regions and design degenerate PCR primers used for cloning and sequencing from five seagrass species: *Halodule beaudettei* (synonymous with *H. wrightii*, Cymodoceaceae), *Cymodocea filiformis* (Cymodoceaceae), *Thalassia testudinum* (Hydrocharitaceae), *Halophila engelmannii* (Hydrocharitaceae), and *Ruppia maritima* (Ruppiaceae). Amplification of the desired stress-related genes from seagrasses was unsuccessful. *Hb1* primers yielded PCR products from *H. beaudettei* around the expected size (~759 bp), but sequence analysis identified this as a bacterial-like NAD/NADP octopine/nopaline dehydrogenase. Using genomic DNA, actin gene fragments (1-1.8 kb) corresponding to exons 2-4 were amplified from five species, and

Gapdh (exons 5-9) was amplified from *H. beaudettei*. Intron length varied for actin with *C. filiformis* containing the largest introns. Splicing junctions were verified comparing cDNA sequences from *H. beaudettei*. Actin and GAPDH sequences were aligned in MEGA using MUSCLE and compared with other plant sequences in GenBank®. A phylogenetic tree was constructed for each gene using Maximum Likelihood with 1,000 bootstrap replicates. Actin cDNA sequences from the same families grouped together to form clades reaffirming phylogeny. However, the genomic sequences of *H. beaudettei* actin, as well as GAPDH, did not group together with the expected clade, unlike the cDNA sequences from the same species. The genomic actin sequences were most closely related to rice *Act1*, which is grouped with reproductive actins in other plants. Similarly, the genomic sequences of GAPDH did not group together with the mRNA sequences, but instead grouped with dicots reaffirming BLAST search results. Mean codon bias differences in genomic sequences vs. cDNA along with differences in theoretical isoeletric points seem to indicate multiple members of gene families for actin and GAPDH in *H. beaudettei*, similar to previous work in all angiosperms studied thus far. This finding suggests that the genomic vs. cDNA clones of both actin and GAPDH may represent differentially expressed paralogs. This work raises the interesting possibility that expression patterns of individual housekeeping paralogs could be used as stress indicators. Future work should include high-throughput sequencing to analyze expressed housekeeping genes under a variety of environmental conditions and to identify stress-related gene candidates in the transcriptome.

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INTRODUCTION

Background and Relevance

Seagrass meadows contribute habitat and primary productivity to estuaries and near-shore marine environments (Hemminga and Duarte, 2000). Conservative estimates of the value of ecosystem services provided by seagrass beds are in the order of $19,000 ha^{-1} yr^{-1} (Costanza *et al*., 1997). However, seagrasses are quite vulnerable, and their growth and productivity are limited by salinity, water clarity, temperature, and nutrient loading (Hemminga and Duarte, 2000; Wyllie-Echeverria *et al*., 2002). Physiochemical conditions may be exacerbated by anthropogenic activity, and climate change is expected to affect both seagrass productivity and distribution (Short and Neckles, 1999).

Eutrophication, one of the most widely reported anthropogenic causes of seagrass decline, is linked with coastal development and reduced water quality (Wyllie-Echeverria *et al*., 2002; Ralph *et al.*, 2006). Anthropogenic nutrient sources include sewage effluent, septic system seepage, storm-water outfalls, industry, aquaculture and agricultural runoff (Ralph et al, 2006). Nutrients increase water column algae and seagrass epiphytes (Duarte, 2005), which are composed of sessile plants and animals, algae, bacteria and fungi that grow attached to seagrasses. Overgrowth of epiphytes, as with water column phytoplankton, can account for losses of submerged aquatic vegetation (Phillips *et al*., 1978; Kemp *et al*., 1983; Cambridge *et al*., 1986) by reducing the absorption of light, gas exchange, and the uptake of nutrients (Sand-Jensen, 1977). Dunton (1996), however, found a persistent dense algal bloom in Laguna Madre, TX (LM) despite relatively low dissolved inorganic nitrogen levels (<5 μM), apparently contradicting a simple

relationship between nitrogen concentration and algal growth. Algae load in the water column, however, has also been correlated with phosphorous levels (Frankovich and Fourqurean, 1997), but this is not considered a limiting factor along the Texas coast (Örnólfsdóttir *et al*., 2004). Moreover, contradictory findings demonstrated that epiphytic load may be determined more by the assemblage of grazers rather than nutrient loading (Heck *et al*., 2000; Hays, 2005) and that epiphytes may not be good indicators of nutrient loading or eutrophication (Lin *et al*., 1996). The emerging picture is that site-specific conditions dictate the prevalence of bottom-up vs. top-down control of epiphyte loads (see Peterson *et al*., 2007), but regardless of the mechanism, excessive nutrients can stimulate epiphyte loads and diminish seagrass status.

Some epiphytes, such as the fungi and protists, can invade plant tissue. For example, *Labyrinthula zosterae*, a protist, and *Lindra thalassiae*, a pathogenic fungus, have been known to cause disease outbreaks in seagrass beds of *Thalassia testudinum* (Short *et al*., 1986; Muehlstein, 1992). *Labyrinthula* was identified as the primary causative agent in the "wasting disease" (Zosteraceae) outbreak during the 1930s and 1940s (Muehlstein, 1989). Secondary decomposers of senescent or stressed seagrasses are suggested to be opportunistically pathogenic (Muehlstein, 1992). Plant-produced phenolic compounds are known to have antimicrobial properties and have been suggested as a microbial barrier for seagrasses against invading microbes (Harborne, 1977; Harrison, 1982). Interestingly, sulphated phenolic acids have been isolated from seagrasses, such as *Halodule*, and may play a role in the adaptation to the marine environment though the ecological significance is not yet clear (McMillan *et al*., 1980).

Production of such antimicrobials may potentially be a stress response to pathogens in epiphytic biofilms.

Seagrasses are limited in their distribution to areas where the sediment is not overly anoxic and sufficient incident light reaches the plants. Unlike fresh water plants that store large amounts of O_2 in gas-filled lacunae, seagrasses do not store amounts of O_2 adequate for more than hours—only minutes in *Zostera marina*. To sustain respiration during times of reduced light or at night, seagrasses must obtain oxygen from the water column (Sand-Jensen *et al*., 2005). If their sediments become anoxic, oxygen must continually leak from roots and rhizomes into anoxic sediments during both light and dark periods to counteract diffusion of reduced phytotoxins (i.e. H_2S) from entering the root system (Borum *et al*., 2006). Lamote and Dunton (2006) found sediment porewater concentrations of sulfides were inversely correlated to light concentration. Therefore, light is a limiting factor for photosynthetic maintenance of adequate levels of O_2 in the roots and rhizomes. The percentage of the incident light reaching a certain depth—an attenuation of the photosynthetically active radiation (PAR) from the surface—can be determined using the Lambert-Beer equation ($A=\varepsilon\ell c$). If surface irradiance (SI) drops to less than 18% for species, including *Halodule wrightii*, in the northwestern Gulf of Mexico, sediment oxygen levels decrease and toxic concentrations of sulfides and ammonium accumulate (Dunton 1994; Mateo *et al*., 2006).

With so many variables, there seems to be no clear relationship to reconcile the complex interactions of biotic and abiotic factors that may limit seagrass productivity, an understanding of which is necessary for formulating a predictive model. Indeed, a central

challenge for biology is predicting species' responses to the environment (Lubchenco, 1998). Measurements of biomass, species abundance and distribution are commonly used to gauge the physiological status of seagrass meadows, but these are "lagging indicators" of the underlying limiting factors and do not clearly reveal the causal factors, despite attempted correlations with environmental parameters. For example, leaf height has been found to be a good indicator for gauging seagrass bed recovery but not as an indicator of impending loss (Onuf and Ingold, 2007). In addition, seagrass cover has been noted to fluctuate in the apparent absence of detectable environmental changes (Onuf and Ingold, 2007). Chlorophyll fluorescence measurements can provide insight into the energetic status of seagrasses (Lamote and Dunton, 2006), but identifying expression patterns of stress-related genes can add additional information on how seagrasses try to maintain homeostasis in a changing environment. Molecular markers can serve as physiological indicators on a more precise scale than the aforementioned endpoint measurements and can be important tools for understanding how a plant responds to the complex interactions with its environmental conditions (Procaccini *et al*., 2007).

The merging disciplines of ecology and genomics, referred to as ecogenomics, may yield a greater understanding about an organism's reaction to the environment by using model organisms such as *Oryza* (rice) to seek the molecular basis of critical traits, such as stress resistance, pathogen defense, herbivore deterrence and life-history in plants. These techniques are now becoming applicable to non-model organisms such as

seagrasses (Procaccini *et al*., 2007), and should give more precise tools to link ecological events to the physiological status of seagrass beds.

The most studied seagrass genera are *Thalassia, Posidonia,* and *Zostera*, which taken together form most of the world's seagrass meadows (Larkum *et al*., 2006). Genes involved in the metabolism of heavy metals and in water transport have been isolated and characterized in *Posidonia oceanica* (Meastrini *et al*., 2004; Cozza *et al*., 2006), and heat shock proteins, which confer metabolic protection via refolding of denatured proteins under temperature stress, are also under investigation in the monocot *Zostera marina* (Boston *et al*., 1996). Housekeeping genes are being identified in *Z. marina* for baseline measurements in real-time quantitative PCR (Ransbotyn and Reusch, 2006). These studies are giving way to the first generation of microarrays in *P. oceanica* (Procaccini *et al*., 2007). However, there is a conspicuous lack of molecular knowledge of seagrass species in the western Gulf of Mexico such as *Halodule beaudettei* (synonymous with *Halodule wrightii*).

The most common seagrass found in all bays along the Texas Gulf Coast is the marine monocot *Halodule beaudettei*, with the most extensive beds occurring in the upper LM (Pulich and White, 1997). This seagrass is important as a habitat for migratory waterfowl, wading and diving birds (i.e. pelicans and loons), and is a food source for redhead ducks, manatees and sea turtles (Texas Department of Parks and Wildlife, 1999). *H. beaudettei* biomass in core samples declined in the LM by >60% over a five-yr period during 1988-1993 (Onuf 1996), most likely caused by the attenuation of light by a persistent brown tide bloom (Dunton, 1996; Whiteledge *et al*., 1999). Other seagrasses

that occur in and around *H. beaudettei* beds are *Cymodocea filiformis* (also known as *Syringodium filiformis*), *Thalassia testudinum*, *Halophila engelmannii* (native to Caribbean waters and considered invasive along the Texas Gulf Coast), and *Ruppia maritima*—a halotolerant freshwater species. Little is known how each species adapts to the conditions in local waters beyond salinity and light requirements ranges. **There is a need to understand the molecular stress response mechanisms of *H. beaudettei* and other seagrasses in the LM to identify precise indicators of environmental stresses such as light attenuation, hypoxia, and nutrient loading.** Identifying stress-related gene expression patterns can illuminate how seagrasses try to maintain homeostasis in a changing environment (Fig. 1). Initial short term responses would typically be altered enzyme activities, while slower, longer term responses invoke changes in gene expression. Some responses are specific to abiotic stresses or biotic stresses, while others are involved in both types of stress as a generalized stress response. This research will lay the groundwork for future studies involving monitoring seagrasses stress responses by molecular techniques.

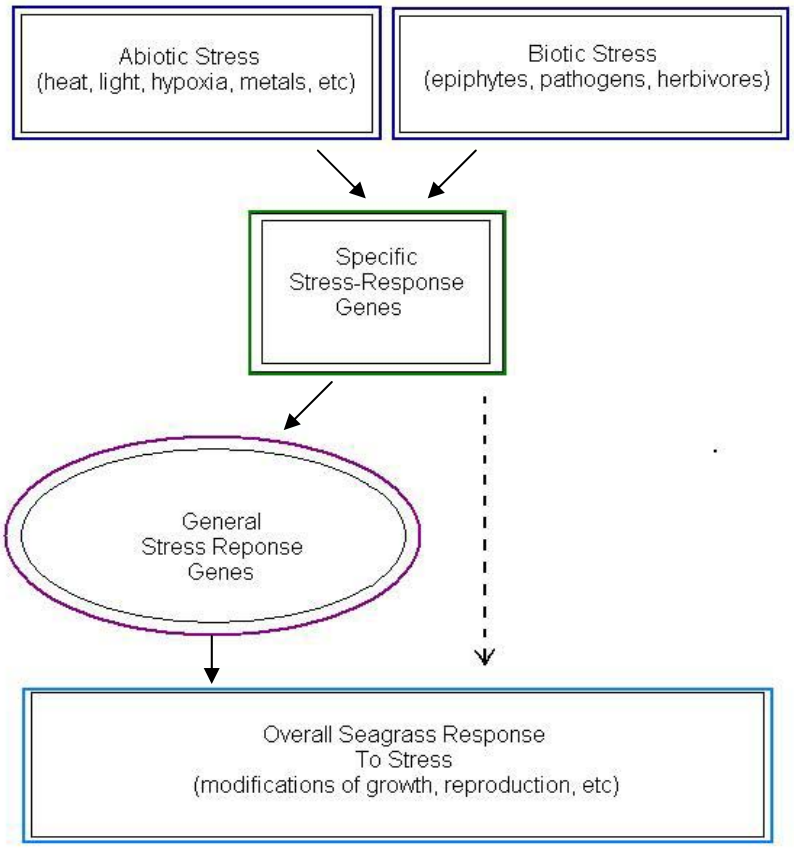

FIG.1. Multiple stressors and stress response pathways in plants.

Literature research has suggested three gene candidates of particular interest: *ascorbate peroxidase 1*(*Apx1*), *hemoglobin 1* (*Hb1*), *and phenylalanine ammonia lyase 1* (*Pal1*). These genes have a role in the responses of plants to biotic and abiotic stresses. The APX1 enzyme protects cells from oxidative damage by playing a key role in scavenging reactive oxygen species (ROS) (Mehdy *et al*., 1996). *Hb1* expression is induced in plant tissues experiencing hypoxic conditions (Igamberdiev *et al*., 2004; Igamberdiev *et al*, 2006). PAL1 is the key regulatory enzyme for the production of phenolic compounds and phytoalexins (Grace, 2005; Boudet, 2007). Production of

antimicrobials would be especially important for seagrasses experiencing environmental stresses (i.e. low light, high temperatures) or high epiphyte loads, which may make them more susceptible to infections as pointed out by Ross *et al*. (2007).

Reactive oxygen species (ROS) are produced during normal cellular metabolism (i.e. the aerobic phase of photosynthesis and photorespiration) but can also be produced in response to environmental stresses (Kotchoni and Gachomo, 2006; Mehdy *et al.*, 1996). Slight changes in homeostatic levels of ROS can trigger expression of antioxidant proteins such as APX1, thereby protecting cells against the toxic effects of ROS. APX1 catalyzes the conversion of H_2O_2 to H_2O and O_2 using ascorbate as the electron donor (Asada, 1999). Various ROS, however, also play a role in plant defenses against invading organisms by directly attacking the invader, strengthening the cell wall by cross-linking of cell wall components (Otte and Barz, 1996), activation of defense genes (Jabs *et al*., 1997), inducing caspase activity (Ge *et al*., 2005), and programmed cell death (apoptosis) to limit pathogenesis (Levine *et al*., 1994; Lamb and Dixon, 1997). It has been recently demonstrated that H_2O_2 is produced in seagrasses at the site of exposure to fungal pathogens (Ross *et al*., 2007). After pathogen challenge, APX1 might be important for restoring low homeostatic levels of ROS.

Hb1, a non-symbiotic class 1 hemoglobin, binds O_2 but can also bind nitric oxide (NO). Under hypoxic conditions, plant mitochondria can use nitrite as an electron acceptor to oxidize cytosolic NADH/NADPH and generate ATP (Stoimenova *et al.*, 2007). NO is a byproduct of this reaction but can be regenerated to nitrite via the enzymatic action of Hb1 with NAD(P)H and nitrate reductase (Fig. 2). When oxygen

concentrations are below that required for saturation of cytochrome *c* oxidase (*COX*), nitrite can serve as an alternative electron acceptor at complex III and COX in plant mitochondria (see Fig 2, Stoimenova *et al.*, 2007), and this would explain why in plants the overexpression of class 1 non-symbiotic hemoglobin was shown to reduce NO levels and protect alfalfa roots under hypoxic conditions (Dordas *et al.*, 2003). Hb1 may also play a role in NO stress signaling under hypoxic conditions (Stoimenova, *et al*., 2007).

NO can also induce PAL1, a key regulatory enzyme for synthesis of salicylic acid (Durner *et al.*, 1998), which plays a critical role in the activation of plant defense responses after pathogen attack (see Klessig *et al*., 2000), and in the synthesis of potentially antimicrobial phenolic compounds. When challenged with fungal pathogens, PAL1 was upregulated in sorghum seedlings (Cui *et al*., 1996). Phenolic compound synthesis is also induced in response to other biotic and abiotic stimuli such as UV-B radiation, drought, chilling, ozone, heavy metals, attack by pathogens, wounding, or nutrient deficiency (Dixon and Paiva, 1995; Grace, 2005). It may be a potential indicator of either abiotic stress due to hypoxic conditions or biotic stress from pathogen attack. Understanding *Pal1* may lead to a better understanding of conditions that influence phenolic compound production as well as a general stress response.

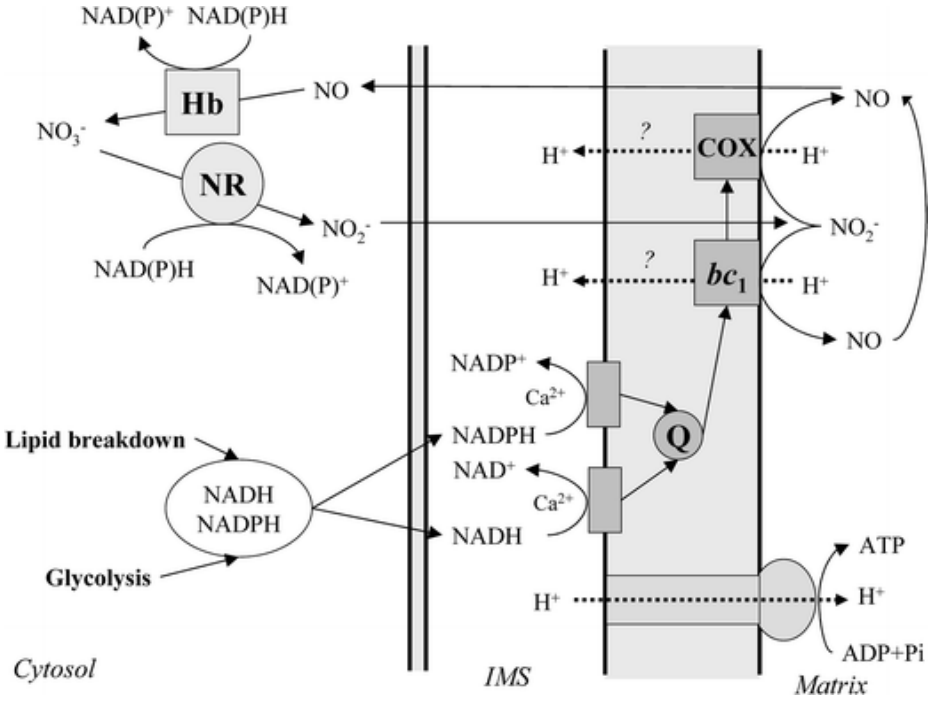

FIG. 2. Operation of plant mitochondria under hypoxic conditions. Glycolytic fermentation and lipid breakdown in hypoxia result in the increase of cytosolic NADH and NADPH. Externally facing Ca2+-dependent mitochondrial dehydrogenases oxidize NADH and NADPH and transfer electrons to ubiquinone (Q). At levels of oxygen below saturation of cytochrome c oxidase (COX), nitrite can serve as an alternative electron acceptor at the sites of complex III and COX. Nitric oxide (NO) formed in this reaction is converted by hypoxically induced hemoglobin (Hb) to nitrate (NO_3^-). The latter is reduced to nitrite (NO_2^-) by hypoxically induced nitrate reductase (NR). ATP is synthesized due to proton pumping possibly at the sites of complex III (bc_1) and COX. IMS = intermembrane space of mitochondria (used with permission from Stoimenova *et al.*, 2007).

I propose to explore the use of expression patterns of these stress-related genes to serve as leading indicators of seagrass stress. My initial hypothesis was that stress-related genes in *H. beaudettei* are expressed as a response to changing environmental conditions, such as eutrophication, or hypoxic conditions. However, these genes first need to be identified in seagrasses by amplification, sequencing, and expression pattern exploration.

In order to conduct gene-expression assays, an internal standard must be used. Housekeeping genes, those that are expressed at a steady state and needed by the cell at all times, are used to help normalize gene expression results between samples. One example of a housekeeping gene is actin. It is involved with cell division and growth, cell polarity and shape, intracellular motility of cytoplasm and organelles (cytoplasmic streaming), cellular responses to external stimuli, extension growth, and cell wall synthesis (Staiger and Schliwa, 1987; Meagher and Williamson, 1994; Mathur, 2004; Wasteneys and Yang, 2004; Smith and Oppenheimer, 2005).

Actin genes in angiosperms belong to large, multigene families ranging from 10 to more than 100 genes (Meagher, 1991; McDowell *et al*., 1996). For example, *Arabidopsis thaliana*— which has the most studied actin family—contains 10 members (two of which are pseudogenes), while rice contains eight members (McElroy *el al*., 1990; McDowell *et al*., 1996). Many plants (*i.e.* soybean, potato, lodgepole pine) contain dozens of actin genes (Meagher, 1991; Meagher and Williamson, 1994). The extreme is petunia with close to 200 actin sequences in its genome (Baird and Meagher, 1987). These genes are thought to have evolved from an ancestral gene that diverged with the emergence of the major tissues and organs in plants. These actins can be divided into two major classes, vegetative and reproductive, with these two classes being further subdivided into five subclasses in *Arabidopsis* (McDowell *et al*., 1996a). Most "reproductive" actins are expressed in reproductive tissues and are not seen in vegetative tissues and visa versa, but there are exceptions. For example, the most abundant rice actin in all tissues and stages is RAc1, which is actually more closely related to other

reproductive actins in other plants rather than other vegetative actins (McElroy *et al.*, 1990).

There is some overlap of expression of different actin family members in plant tissues with varying expression of particular actin genes, but the sum of actin transcripts in the cell does not change much because of their abundance and buffering effect due to the presence of multiple differently responding genes (Klyachko, 2006). The proposed reason for the coexpression of multiple actin isovariants in the same cell, resulting in isodynamics, is that it allows for more complex cytoskeleton responses permitting plants to better adapt to spatial and temporal changes (Meagher *et al.*, 1999a).

Gene families are quite common in plants, including other cytoskeleton proteins, various enzymes, and regulatory/signal transduction proteins (Meagher *et al.*, 1999a). Glyceraldehye-3-phosphate dehydrogenases (GAPDH) are also likely to be coded by a multigene family. For example, corn appears to have three and possibly four cytosolic forms of GAPDH (Russell and Sachs, 1989; Russell and Sachs, 1991). *Amsinckia spectabilis* was found to have at least three members of the *GapC* gene family (Pérusse and Schoen, 2004). *GapC* genes code for enzymes (EC 1.2.1.12) that are involved in glycolysis (catabolism) and are formed from either homotetramers or heterotetramers, if the genome codes for more than one cytosolic form (Cerff, 1982; Russell and Sachs, 1991). This enzyme binds NAD(H) and not NADP(H), in contrast to the chloroplastic GAPDH (EC 1.2.1.13) involved with the Calvin cycle (anabolism) (Cerff, 1978), which preferentially binds NADP(H) and is encoded by nuclear genes *GapA* and *GapB* genes (Cerff, 1978; Cerff and Chambers, 1979). The plastid form is induced by light *in vivo*

(Cerff and Chambers, 1979; Cerff and Kloppstech 1982; Kwon *et al*., 1995; Park *et al*., 1996).

Both forms of the enzyme catalyze the reversible reduction of 1,3-bisphosphoglycerate to glyceraldehyde-3-phosphate. Cytosolic forms are generally thought of as housekeeping genes; however, some forms can be stimulated by environmental stress factors such as anaerobiosis, heat shock, and salinity stress (Martinez *et al.*, 1989; Russell and Sachs, 1989; Yang *et al*., 1993). *Arabidopsis* only contains one copy of the cytosolic form, and this form can be upregulated in tissues with high metabolic demand (Yang *et al*., 1993).

Several studies have elucidated expression patterns of actin and GAPDH in model organisms and economically important crops. Neither gene family has been studied in seagrasses, so many questions remain. How big are these families in seagrasses? Do they mirror most angiosperms with multiple members in each family? If multiple genes are found, do they differ in their isoelectric points that would indicate cytosolic isovariant dynamics? This study will also attempt to identify new members of these families and to examine expression of these genes in *H. beaudettei* with the goal of establishing control housekeeping gene candidates for expression studies.

These goals are important, because the population along the Texas coast is expected to more than double in the next 20 years and the anthropogenic impacts in the local bays and estuaries will increase. The identification of seagrass stress genes will lead to quantitative assays (e.g. real time PCR-qPCR) to enable researchers to quickly recognize and further characterize conditions that induce expression of these genes.

MATERIALS AND METHODS

Identification of candidate stress response genes and development of PCR primers

A literature search was conducted to identify stress-related genes using the rice (*Oryza sativa*) genome as a model. In addition, a search was conducted for candidate housekeeping genes for standardizing future gene expression experiments. Amino acid sequences of genes of interest were collected at the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/) and Swiss-Prot (http://www.expasy.org/sprot/). A BLAST search (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi), using default parameters in BLASTP 2.2.18 (Altschul *et al*., 1997), was used to identify corresponding proteins in other species for multiple sequence alignments. Multiple amino acid sequence alignments were constructed (Appendix A) to identify conserved regions in these stress-related proteins using TCOFFEE (O'Sullivan *et al*., 2004) with the EXPRESSO option when 3-D structures were available (http://www.tcoffee.org/). CLUSTALW (Thompson *et al*., 1994) was used if sequence lengths exceeded TCOFFEE limits. If there was a substantial amount of conservation in the amino acid sequences, nucleotide sequence alignments using TCOFFEE or CLUSTALW were used to further assess conserved regions. Multiple nucleotide sequence alignments with at least 15 consecutive nucleotides of conservation in sequences were used to design degenerate primers using Primaclade (Gadberry *et al*., 2005) available at the University of Missouri-St. Louis website (http://www.umsl.edu/services/kellogg/primaclade.html). The final candidate genes chosen are listed in Table 1.

TABLE 1. *List of candidate stress-related and housekeeping (control) genes*

Gene	Function	Reference
Pal1	Key regulatory enzyme for phenolic compound production	Harborne, 1977; McMillan *et al*., 1980
Apx1	Protects cell against ROS; produced under environmental stresses and pathogenic attack	Kotchoni and Gachomo, 2006; Mehdy *et al*., 1996
Hb1	Involved with hypoxic mitochondrial respiration; binds NO; converts NO → NO_3^-	Igamberdiev *et al*., 2004; Igamberdiev *et* al., 2006; Stoimenova, *et al*., 2007
Act1	Part of cytoskeleton, responsible for organelle movement and various other cellular processes including cell division	McCurdy *et al*., 2001; Jain *et al.,* 2006
Gapdh	Carbohydrate metabolism; 6th step of glycolysis	Bio-Rad GAPDH PCR Module (Hercules, CA)

OligoAnalyzer 3.1 (http://www.idtdna.com/analyzer/Applications/OligoAnalyzer/) was used to analyze primer sequences to assess the following parameters: percentage GC content, T_m, dimerization, and if common motifs (e.g. a DNA or ATP binding region) were present (using NCBI BLAST). Primer sets were selected based on compatibility of selected parameters (Table 2).

TABLE 2. *List of primers developed from nucleotide alignments*

Gene	Forward Primer	Reverse Primer	Expected Amplicon Size (bp)
Pal1-1	5'-GACAKYTACGGYGTCACCA-3'	5'-GGCTTGCCGTTCATVACYT-3'	1877
Pal1-2	5'-ARGTBATGAACGGCAAGCC-3'	5'-VCCRTTGTTGTAGAASTCGTT-3'	440
Pal1-3	5'-GCCTCSTACHGCTCYGAGCT-3'	5'-GCCGTYCCACTCCTTGAGGCA-3'	688
Apx1	5'-TCATYGCSGAGAAGARCTG-3'	5'-CTGGTASARATCGGCGTA-3'	312
Hb1	5'-AKGCGCTGGTGCTCAAGTC-3'	5'-GGCTTCATCTCYYGCTTGAT-3'	759
Act1	5'-GCATCACACYTTCTACAAYGAG-3'	5'-TTAGAAGCYTTCCTGTG-3'	1199

Degenerate bases: K = G,T R = A,G S = C,G Y = C,T B = C,G,T H = A,C,T V = A,C,G. Three different primer sets were designed for *Pal1*: *Pal1*-1, *Pal1*-2, *Pal1*-3. Expected amplicon sizes based on rice.

Internal primers were designed as needed (Table 3). Predicted size of each amplicon was calculated by analyzing rice genomic sequences. For *Gapdh*, initial PCR with nested primers for cytosolic *Gapdh* was performed using the Bio-Rad GAPDH PCR Module (kit #166-5010EDU, Bio-Rad, Hercules, CA).

TABLE 3. *List of primers developed for internal bi-directional sequencing*

Gene-Species	Forward Primer	Reverse Primer
Act1-H.b.-1	5'-GTGCGCTCACGTCGTCTTGT-3'	5'-TGAGCACGATGTYGCCGTAGA-3'
Act1-H.b.-2	5'-CTGTTCCAGCCCTCCATGA-3'	5'-CGGAGTCGAGCACGATACCT-3'
Act1-C.f.-1	5'-GTCGCACAACTGGTAAGCAATA-3'	5'-GATCCACCACTAAGCACGATA-3'
Act1-C.f.-2	5'-CTGACTGATGTTATGAGATGGA-3'	5'-ATGTGGCAGTGCGTATCCTTCA-3'
Act1-H.e.	5'-GCCTCSTACHGCTCYGAGCT-3'	5'-GCCGTYCCACTCCTTGAGGCA-3'
Act1-T.t.	5'-TCTGACGGACTGCTTGATGA-3'	5'-GGCTTAGGTCAAGAGGGTTAG-3'
Act1-R.m.	5'-GGACTCTGGTGATGGTGTTACTC-3'	5'-CTGATATCCACGTCAGACTTCATG-3'
Gapdh-H.b.-1	5'CTACTGGTGTCTTCACTGAC-3'	5'-CAAAGATGCTCGACCTGTTGT-3'
Gapdh-H.b.-2	5'-GGAATTGTTGAGGGTCTTATGA-3'	5'-CTCTTCCACCTCTCCAGTC-3'

Differing pairs of primers for same species are differentiated by numbers. *Gapdh* cDNA sequencing used *Gapdh-H.b.*-1 primer set resulting in a 12 bp shorter amplicon at the 3'-end end vs. the genomic sequence.

Sample collection

Rhizome tissue samples were collected for each seagrass from the northwestern shore of the Upper Laguna Madre and Wilson's Cut, off of Corpus Christi Bay (16.55 km distance between the sites) during July 2008-August 2010. GPS coordinates for sites of collection are given in Appendix C. Note that two different sites were used for tissue sampling of *H. beaudettei*, respectively, for genomic DNA isolation and RNA isolation due to inaccessibility of the former site.

Isolation of genomic DNA and optimization of PCR conditions

DNA from each seagrass (*Halodule beaudettei*, *Cymodocea filiformis*, *Halophila engelmannii*, *Thalassia testudinum*, *Ruppia maritima*) was extracted using the Qiagen

DNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA). The standard Qiagen protocol was adjusted to maximize DNA concentrations. Frozen tissue (100 mg) was used instead of fresh tissue (desiccated leaf blades for *T. testudinum*), placed in a FastPrep® Lysing Matrix A 2.0 mL tube (Qbiogene Inc., Irvine, CA), and sandwiched in between two ceramic beads along with 600 μL of AP1 buffer (Qiagen DNeasy kit). Samples were processed in a FastPrep®-24 homogenizer (MP Biomedicals LLC, Solon, OH) for 40 seconds at 5.0 M/s. *T. testudinum* leaf tissue was homogenized for 2 cycles for 60 seconds at 6.0 M/s followed by horizontal shaking for 15 minutes. Following centrifugation of lysates for 15 min. at 14,000 x g at room temperature, the supernatant was transferred to a clean 1.7 mL microfuge tube and the standard Qiagen DNeasy protocol for DNA isolation used afterwards. DNA concentrations ranged from 20-90 ng/ul. PCR conditions were optimized using Taq-&Go™ Mastermix (MP Biomedicals LLC, Solon, OH) for amount of genomic DNA, [Mg^{2+}], annealing temperature, primer concentration, and the number of cycles of PCR amplification. *Act1* PCR conditions using 100 ng of genomic DNA were 4 min at 94°C; 35 cycles of 1 min at 94°C, 1 min at 55°C, and 2.5 min at 72°C; 5 min at 72°C. *Hb1* PCR conditions using 100 ng of genomic DNA were 4 min at 94°C; 35 cycles of 1 min at 94°C, 1 min at an annealing temperature range of 44.5-49.5°C, and 2.5 min at 72°C; 5 min at 72°C. For *Gapdh*, the Bio-Rad GAPDH PCR Module (Hercules, CA) protocols were used for both initial and nested PCR. Initial conditions for *Gapdh* PCR using 100 ng of genomic DNA were 5 min at 95°C; 40 cycles of 1 min at 95°C, 1 min at 52°C, and 2 min at 72°C; 6 min at 72°C.

Nested PCR conditions were 5 min at 95°C; 40 cycles of 1 min at 95°C, 1 min at 46°C, and 2 min at 72°C; 6 min at 72°C.

Cloning and sequencing

PCR products were cloned into pCR® 2.1-TOPO® vectors using the TOPO TA Cloning Kit containing chemically competent TOP10 *E. coli* cells (Invitrogen Corp., Carlsbad, CA). Clones were selected by blue-white screening, and inserts were analyzed by PCR with M13 primers targeted to vector DNA flanking insert. After screening by agarose gel electrophoresis, PCR products were cleaned using the Qiagen PCR Purification Kit (Qiagen Inc., Valencia, CA). DNA sequencing reactions used the GenomeLab™ DTCS Quick Start Kit (Beckman Coulter, Inc., Brea, CA) and the Beckman Coulter CEQ 8800 Series Genetic Analysis System. Sequence data was assembled with Sequencher (Gene Codes Corp., Ann Arbor, MI) and assessed using BLAST (Altschul *et al*., 1997) searches for orthologs as verification of sequence identity. Internal sequencing primers were designed using the DNASIS Smart Note website (http://smartnote.miraibio.com/) and OligoAnalyzer 3.1 (http://www.idtdna.com/analyzer/Applications/OligoAnalyzer/) to complete bidirectional sequencing. Sequences were annotated using BankIt (NCBI) and submitted to the NCBI (http://www.ncbi.nlm.nih.gov). GenBank Accession numbers and information on sequences can be found in Appendix B.

Tissue sample processing and RNA isolation

H. wrightii rhizomes and blades were collected from study sites and stored immediately in RNA*later*® (Ambion, Applied Biosystems, Austin, TX) according to manufacturer's

instructions. Preserved tissue samples were scraped to remove epiphytic growth and stored at -70 °C in RNA*later*® until processed. Total RNA was isolated from 100 mg of tissue frozen and ground in a pre-chilled mortar and pestle, using the UltraClean Plant RNA Isolation kit (Mo Bio, Carlsbad, CA) along with Plant RNA Isolation Aid (Ambion, Applied Biosystems, Austin, TX), and treated with DNase I (Ambion, Applied biosystems, Austin, TX) according to each manufacturer's instructions. RNA quality was assessed by visualizing banding patterns of rRNA on a 1% agarose gel in 1X TBE.

First-strand cDNA synthesis and PCR

Total RNA was reverse-transcribed using GoScript™ Reverse Transcription System (Promega, Madison, WI) with anchored oligo $(dT)_{23}$ primers (Sigma, St. Louis, MO) for first-strand synthesis using manufacturer's instructions with a 42 °C extension temperature. Subsequent PCR conditions of amplification of *Act1* were 4 min at 94°C; 36 cycles of 1 min at 94°C, 1 min at 55°C, and 2.5 min at 72°C; 5 min at 72°C. For *Gapdh*, the amplification program was 5 min at 95°C; 40 cycles of 1 min at 95°C, 1 min at 46°C, and 2 min at 72°C; 6 min at 72°C. The same primer sets were used for cDNA amplification as were used for genomic DNA amplification.

Cloning and sequencing cDNA

PCR products from cDNA were cloned into pCR® 2.1-TOPO® vectors using the TOPO TA Cloning Kit containing chemically competent TOP10 *E. coli* cells (Invitrogen Corp., Carlsbad, CA). Clones were chosen by blue-white screening, and inserts were analyzed by PCR with M13 primers targeted to vector DNA flanking insert. Following analysis by

agarose gel electrophoresis for correct size, the PCR products were cleaned using the Qiagen PCR Purification Kit (Qiagen Inc., Valencia, CA). DNA sequencing reactions used the GenomeLab™ DTCS Quick Start Kit (Beckman Coulter, Inc., Brea, CA) and the Beckman Coulter CEQ 8800 Series Genetic Analysis System. Alternatively, some sequencing was contracted out to MCLAB (San Francisco, CA). Sequence data was assembled with Sequencher (Gene Codes Corp., Ann Arbor, MI) and assessed using BLAST (Altschul *et al*., 1997) searches for orthologs as verification of sequence identity.

Bioinformatics analyses

Sequences were compared using Sequence Alignment Highlighter (Kuiken *et al.*, 2003), found at http://www.hiv.lanl.gov/content/sequence/HIGHLIGHT/highlighter, to highlight differences in silent vs. non-silent mutations and to compare sequence similarity. Coding regions for protein products were analyzed for predicted molecular weight and isoelectric point (pI) using ProtParam tool at the ExPASy Proteomics Server (http://ca.expasy.org/tools/protparam.html). Codon bias was analyzed applying *Oryza sativa* as the basis for the codon table comparison at the Graphical Codon Usage Analyser (http://gcua.schoedl.de/seqoverall_v2.html), trimming sequences to remove degenerate primer bases and to synchronize with the reading frame for analysis.

Constructing phylogenetic trees

Orthologs were gathered using BLAST and imported from GenBank into *MEGA* version 5 (Tamura *et al.*, 2011) using the translated amino acid sequence from exons. All sequences were trimmed to similar lengths. Nucleotide sequences were aligned using the

MUSCLE (Edgar, 2004) option in *MEGA* version 5 (Tamura *et al.*, 2011). Alignments were then analyzed for the best fit model. Phylogenetic trees were constructed using Maximum Likelihood with the best fit model applying 1,000 bootstrap replications.

RESULTS

Identification of candidate stress response genes and development of PCR primers

Candidate stress-related and control genes were chosen based on physiological roles and representation in the rice genome (Table 1). *Phenylalanine ammonia lyase 1* (*Pal1*) is the key regulatory enzyme for secondary metabolites including sulphated phenolic acids, which are thought to have antimicrobial properties in seagrasses (Harborne, 1977) and to play a role in adaptation to the marine environment (McMillan *et al*., 1980). *Ascorbate peroxidase 1* (*Apx1*) is produced as a protective measure against reactive oxygen species (ROS) and is expressed as a response to abiotic and biotic stressors (Kotchoni and Gachomo, 2006; Mehdy *et al*., 1996). Non-symbiotic *Hemoglobin 1* (*Hb1*) is suspected to play a role in NO stress signaling under hypoxic conditions by binding to NO, which is a byproduct of hypoxic mitochondrial respiration (Stoimenova, *et al*., 2007).

For a housekeeping gene to be used as a control for gene-expression assays, *Actin 1* (*Act1*), part of the cytoskeleton, was chosen because it was constitutively expressed in rice and previously used as a control in gene expression studies (Jain *et al*, 2006). *Glyceraldehyde 3-phosphate dehydrogenase* (*Gapdh*) was also chosen because the

enzyme is involved in glycolysis and primers specific for the cytoplasmic isoform in plants had been developed (Bio-Rad, Hercules, CA).

Amino acid sequence alignments of candidate genes revealed various highly conserved regions in *Pal1* and *Act1* with many consecutive amino acids conserved (Appendix A). *Hb1* and *Apx1* alignments exhibited relatively less conservation with fewer consecutive conserved residues. Regions used for primer development are highlighted in boxes. For nucleotide alignments, monocot sequences were used preferentially over dicot sequences. Alignments of candidate genes revealed regions for a set of primers in *Act1* and 3 sets of primers in *Pal1* (Appendix A), which are highlighted in boxed regions. *Hb1* and *Apx1* alignments each had at least two regions where primers could be developed. A list of primers developed is given in Table 2. Degenerate bases had to be added to address the variances of nucleotides in certain locations in sequence alignments. Degenerate bases have two or more bases that differ at a certain location and appear as a mixture in primer stock.

Isolation of genomic DNA and optimization of PCR conditions

Seagrass rhizomes were collected and used as a source for genomic DNA in order to avoid algal epiphytes attached to the seagrass blades. Genomic DNA concentrations were typically ~20ng/uL except for *T. testudinum*, which yielded concentrations of ~90 ng/uL.

Amplification of *Pal1* from *H. beaudettei* with three different primer pairs resulted in multiple products of various sizes, prompting attempts to optimize PCR conditions. A representative example of optimization is shown in Fig 3. A list of varying

conditions for each primer set can be found in Appendix B (Table V). Expected amplicon products (see Table 2) were not observed for any of the *Pal1* primer sets. Optimization was not reached for any of the *Pal1* primer sets, which gave multiple products (Fig. 3, lanes 2-4) with additional problems with the forward primer priming in both directions revealed through sequencing the largest band, 1.2 kb, which was closest to the 1.8 kb expected size based on rice. The strongest band using the *Pal1*-2 primer set was ~500 bp with an expected amplicon size of 440 bp (Fig. 3, lanes 5-10), but the forward primer from this set also primed in both directions, as revealed through DNA sequencing. PCR with the *Pal1*-3 primer set required 4m*M* Mg^{2+} in the PCR reaction (Fig. 3, lanes 11-15). The expected amplicon size for *Pal1*-3 was 688 bp, and there were two bands about 600 bp, but the reverse primer for *Pal1*-3 was revealed to prime in both directions.

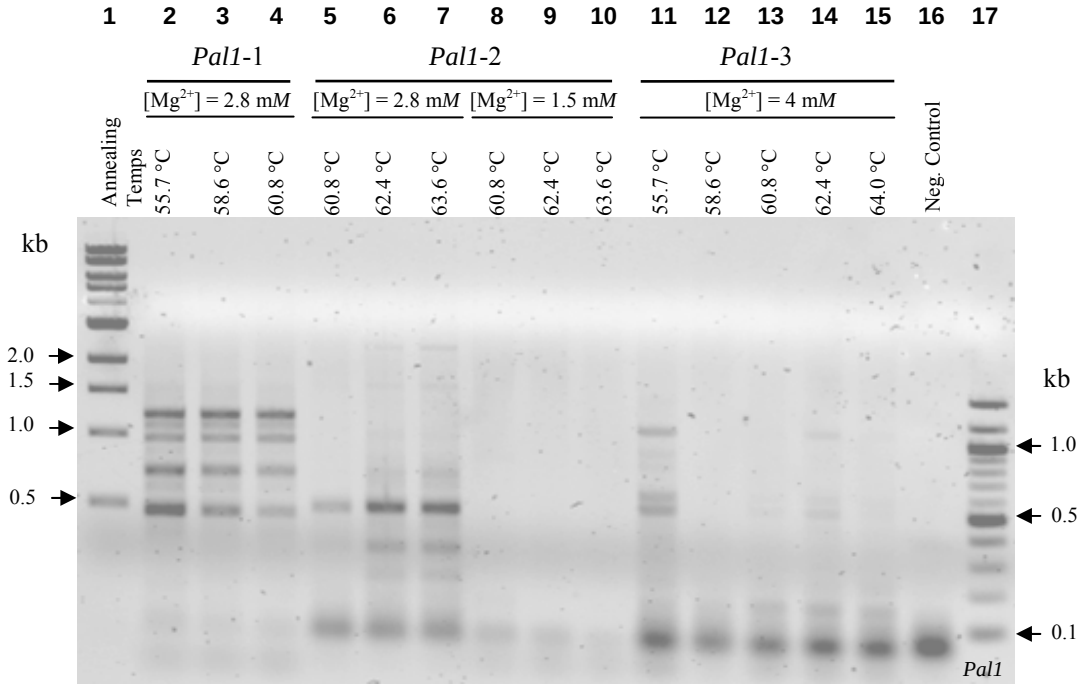

FIG. 3. PCR of *Pal1* using three different primer sets for *H. beaudettei* analyzed on 1% agarose gel. 1kb ladder (lane 1) and 100 bp ladder (lane 17) were used for size standards with a negative control lacking template DNA (lane 16). Varying PCR conditions using *Pal1*-1: 55.7 °C and 2.8 m*M* Mg^{2+} (Lane 2), 58.6 °C and 2.8 m*M* Mg^{2+} (Lane 3), 60.8 °C and 2.8 m*M* Mg^{2+} (Lane 4); *Pal1*-2: 60.8 °C and 2.8 m*M* Mg^{2+} (Lane 5), 62.4 °C and 2.8 m*M* Mg^{2+} (Lane 6), 63.6 °C and 2.8 m*M* Mg^{2+} (Lane 7), 60.8 °C and 1.5 m*M* Mg^{2+} (Lane 8), 62.4 °C and 1.5 m*M* Mg^{2+} (Lane 9), 63.6 °C and 1.5 m*M* Mg^{2+} (Lane 10); *Pal1*-3: 55.7 °C and 4 m*M* Mg^{2+} (Lane 11), 58.6 °C and 4 m*M* Mg^{2+} (Lane 12), 60.8 °C and 4 m*M* Mg^{2+} (Lane 13), 62.4 °C and 4 m*M* Mg^{2+} (Lane 14), 64.0 °C and 4 m*M* Mg^{2+} (Lane 15). Expected amplicon sizes: *Pal1*-1, 1.8 kb; *Pal1*-2, 0.4 kb; *Pal1*-3, 0.69 kb.

There were also amplification issues with degenerate primers designed for the other stress-related genes. The *Apx1* primer set yielded multiple PCR products, decreasing in yield as the annealing temperature was increased, but the expected amplicon size of 312 bp never increased in yield as conditions became more stringent. Less yield was obtained with the PCR enhancer betaine and/or increased annealing temperatures (Fig. 4). It was later revealed through other PCR experiments (data not shown) that both forward and reverse primers primed when used individually; thus expected PCR amplicons were not obtained.

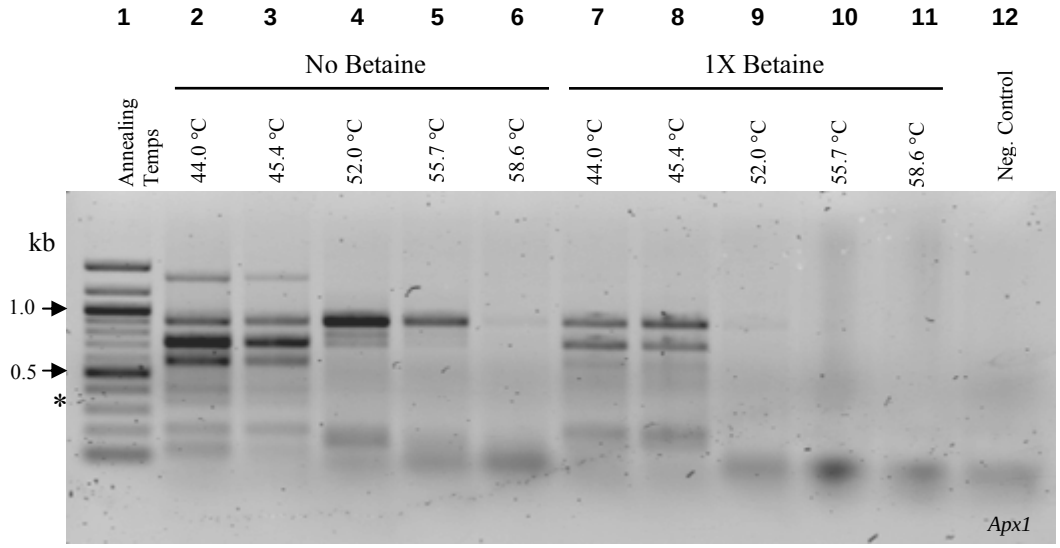

FIG. 4. PCR of *Apx1* from *H. beaudettei* on 1% agarose gel. Size standard: 100 bp ladder (lane 1); negative control lacking template DNA (lane 12). Annealing temperatures: 44 °C (lane 2), 45.4 °C (lane 3), 52 °C (lane 4), 55.7 °C (lane 5), 58.6 °C (lane 6). Betaine as a PCR additive (lanes 7-11). * Expected PCR product sizes based on rice = 0.3 kb.

Similarly, *Hb1* primers amplified multiple products (Fig 5), but those amplicons less than 500 bp were ruled out since the expected coding sequence without introns—a total of three introns based on rice—is 501 bp, and the expected total size based on rice is 759 bp. Amplification products between 700-800 bp were seen at various annealing temperatures, but no optimization was reached when temperature, [Mg^{2+}], and cycling parameters were varied (data not shown). The *Hb1* band at the expected size was cloned and sequenced. BLAST sequence comparison results revealed similarity to bacterial NAD/NADP octopine/nopaline dehydrogenases.

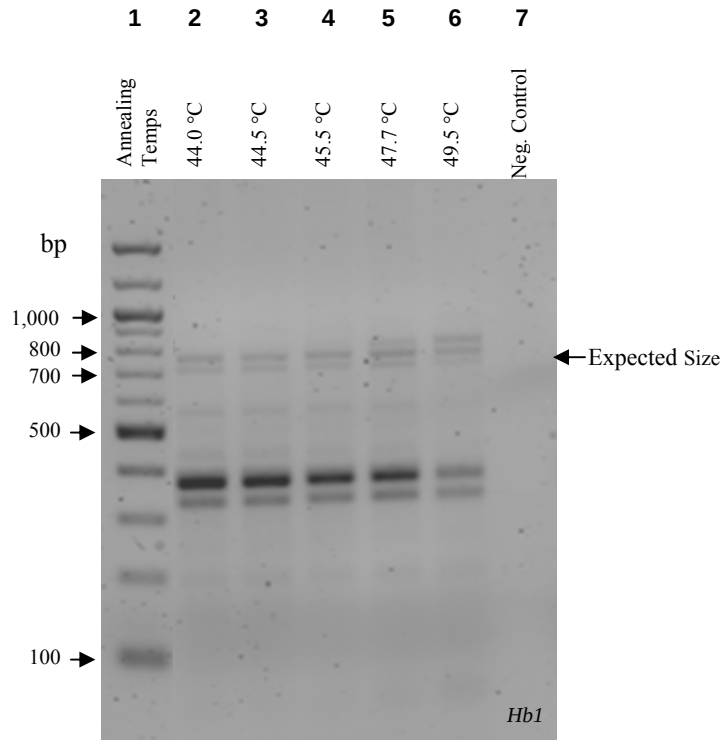

FIG. 5. PCR of *Hb1* in *H. beaudettei* on 2% agarose gel. Size standard: 100 bp ladder (lane 1); negative control lacking template DNA (lane 7). Annealing temperature: 44 °C (lane 2), 44.5 °C (lane 3), 45.5 °C (lane 4), 47.7 °C (lane 5), 49.5 °C (lane 6). Expected PCR product size based on rice = 759 bp.

In contrast to difficulties with the stress-related genes, amplification of *Act1* was accomplished by varying PCR conditions. Various annealing temperatures and Mg^{2+} concentrations (1.5-4 m*M*) were explored (Fig. 6) in order to optimize PCR for *Act1*. Amplification was sharply dependent on Mg^{2+} concentration. Optimal conditions for *Act1* amplification from *H. beaudettei* included a range of annealing temperatures (52-55 °C) and 2.1 m*M* Mg^{2+}. The amplicon size (~1.2 kb) was similar to the size of the *Act1* amplicon predicted from the rice genome.

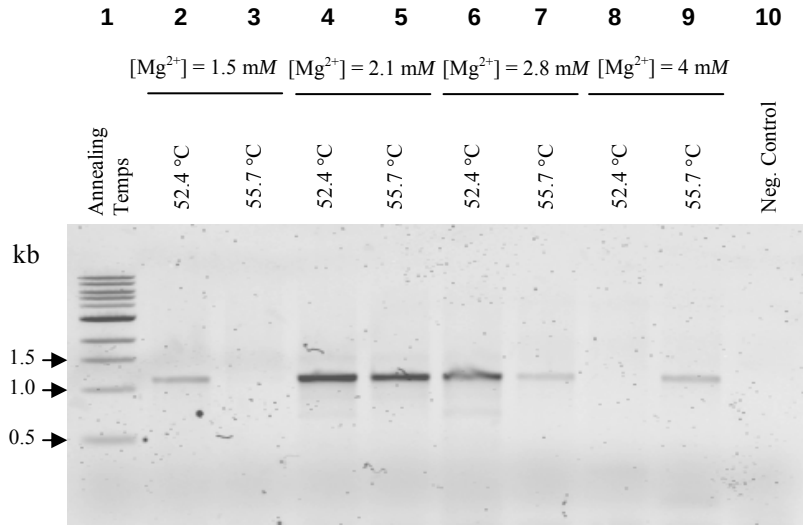

FIG. 6. PCR Optimization for amplification of *Act1* from *H. beaudettei* analyzed on 1% agarose gel. 1 kb ladder (Lane 1). Various annealing temperatures and [Mg^{2+}] were explored: 52.4 °C and 1.5 m*M* Mg^{2+}(Lane 2), 55.7 °C and 1.5 m*M* Mg^{2+} (Lane 3), 52.4 °C and 2.1 m*M* Mg^{2+} (Lane 4), 55.7 °C and 2.1 m*M* Mg^{2+} (Lane 5), 52.4 °C and 2.8 m*M* Mg^{2+} (Lane 6), 55.7 °C and 2.8 m*M* Mg^{2+} (Lane 7), 52.4 °C and 4 m*M* Mg^{2+} (Lane 8), 55.7 °C and 4 m*M* Mg^{2+}(Lane 9), negative control lacking template DNA (Lane 10). All PCR reactions used 100 ng of genomic DNA template and cycling conditions as described in Methods.

The same PCR conditions were applied to amplify actin from the other seagrasses yielding products (Fig. 7) ranging from 1kb-1.8 kb. *C. filiformis* had the largest amplicon size (1.8 kb), but there was also a product (~800 bp) that could be a pseudogene comprised only of exons (coding size of rice *Act1* = 871 bp) or non-specific priming. Alternatively, it may have resulted from a non-seagrass DNA. A sequenced *C. filiformis* clone derived from a ~800 bp amplicon had similarity to *Drosophila melanogaster* actin sequences in BLAST searches with 100% of the query being matched. This product was probably due to contaminating invertebrate DNA and was not investigated further. Other products, in addition to the expected target size, were seen for *H. engelmannnii*, *T. testudinum*, and *R. maritima*, but these were only minor products compared to the expected amplicons and probably resulted from non-specific priming. *R. maritima* had

the smallest amplicon (~1 kb) with *H. engelmannii* (~1.1 kb) and *T. testudinum* (~1.3 kb) having intermediately-sized products.

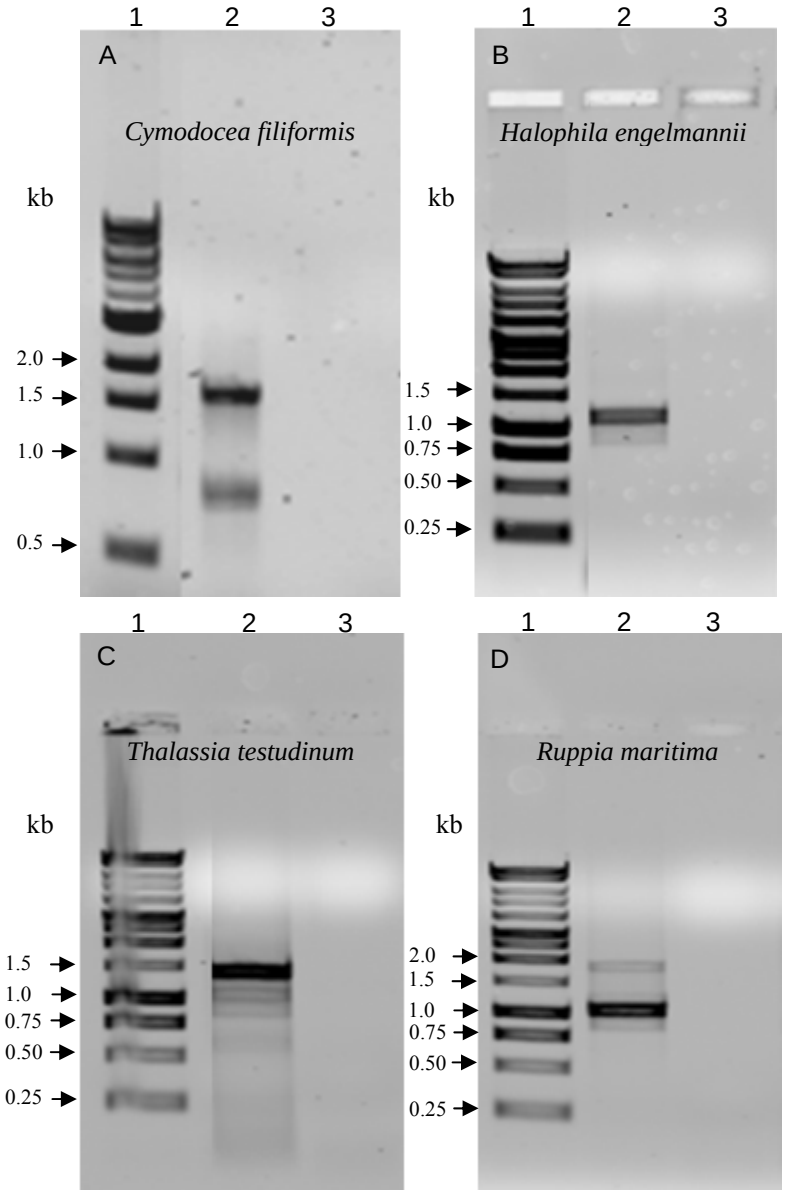

FIG. 7. *Actin* PCR from seagrasses analyzed on 1% agarose gels. Amplification conditions as described for *H. beaudettei* in Methods was used for the other seagrasses: *Cymodocea filiformis* 1.8 kb product (A), *Halophila engelmannii* 1.1 kb product (B), *Thalassia testudinum* 1.3 kb product (C), *Ruppia maritima* 1 kb product (D); 1 kb ladder (lane 1), *Act1* PCR product (lane 2), and negative control lacking template DNA (Lane 3).

Amplification of *Gapdh* was achieved from *H. beaudettei* (Fig. 8) using a two-step nested PCR method (Bio-Rad). The initial *Gapdh* PCR had no visible product for *H. beaudettei* (Fig. 8, lane 3) near the expected size 1 kb, but according to manufacturer's instructions, it is not unexpected to see bands only after the second-round nested PCR (Bio-Rad, Hercules, CA). The positive control also had only a very light band near the 1 kb size (Fig. 8, lane 2). The light band between 100 and 200 bp was most likely due to primer-dimers. Following the second, nested PCR reaction, a ~1 kb band near the expected size of 993 bp was seen in both the positive control and *H. beaudettei*.

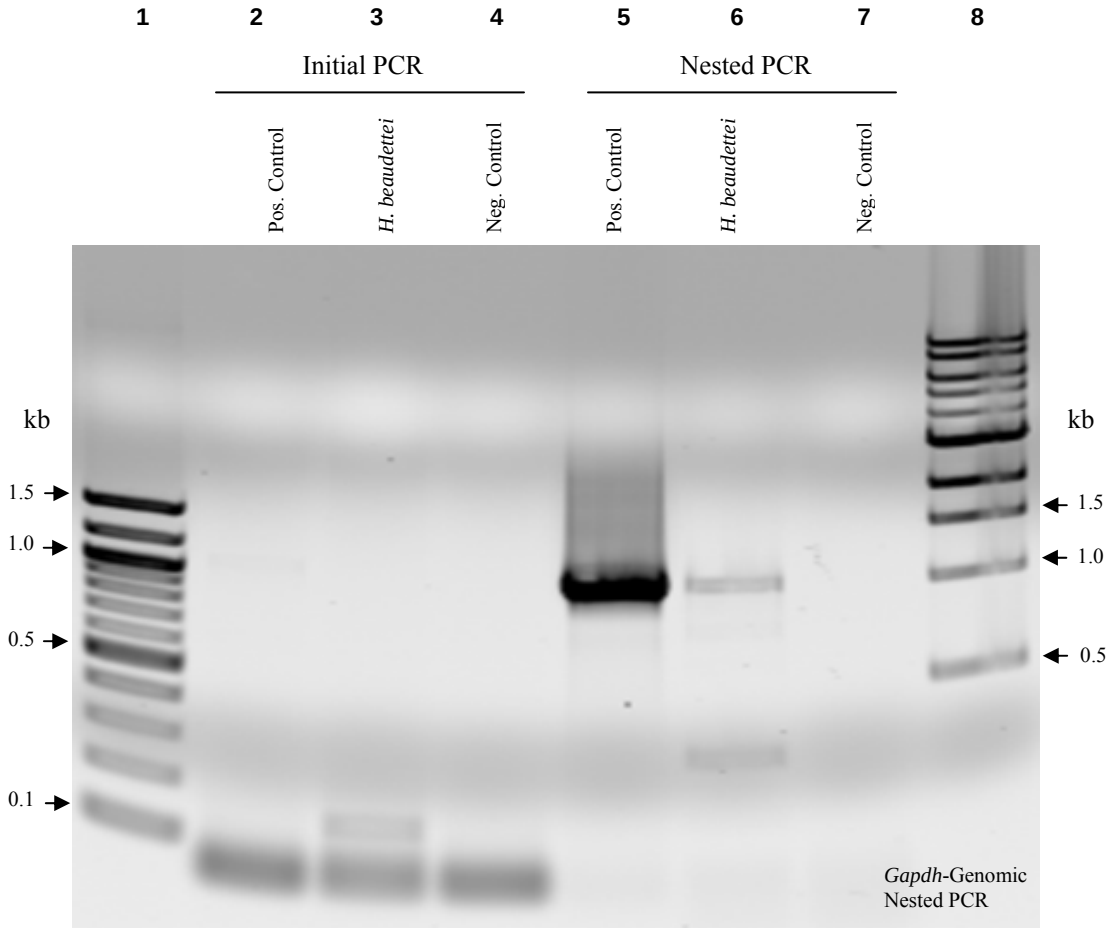

FIG. 8. *Gapdh* initial and nested PCR from *H. beaudettei* analyzed on 1% agarose gel. Size standard: 100 bp ladder (lane 1) and 1 kb ladder (lane 8); negative control lacking template DNA (lanes 4 & 7). Initial PCR: Positive control (*Arabidopsis thaliana*) (lane 2), *H. beaudettei* (lane 3), negative control (lane 4). Nested PCR: positive control (*A. thaliana*) (lane 5), *H. beaudettei* (lane 6). Predicted Nested PCR product size = 993 bp.

Cloning and Sequencing

PCR amplicons from putative stress and control genes were cloned into pCR® 2.1-TOPO® vectors for sequencing from primers targeting the flanking vector DNA. To complete bidirectional sequencing, internal primers were created from initial sequence results (Table 3). *C. filiformis Act1* required two internal sets of primers due to its length (Table 3). Two internal primer sets for *Act1* in *H. beaudettei* were also used due to short

sequence reads, but all other species were internally sequenced in the *Act1* gene through ABI (Carlsbad, CA) sequencing technology (contract through MCLAB (San Francisco, CA), which resulted in longer sequence reads. Because of length difference (~400 bp) between the genomic sequence and the cDNA developed from mRNA, separate internal primer sets were developed for *Gapdh* genomic clones.

The *Act1* gene length varied (1-1.8 kb) by seagrass species (Table 4) due to differing intron lengths. Species within the same seagrass families also differed. *C. filiformis* had the largest intron two sequence, approximately eight times the size of that of the other seagrass sequences, while *T. testudinum* had the largest intron 3 sequence, approximately four times the size of any of the other sequences. Intron sequences for all genomic actin sequences contained conserved GT and AG splicing sequences (data not shown). Coding sequence (exons) similarity to rice also varied between 80-85% with *H. beaudettei* having the most similarity with rice. *H. engelmannii* and *R. maritima* were the two seagrasses that had the least similarity with rice.

Comparable results were seen with *H. beaudettei Gapdh*. Nucleotide similarity with rice *Gapdh* was 85%, the same as for *Act1* (Table 5). Intron length comprised about 39% (387/993) of the amplified sequence and contained conserved GT and AG splicing sequences (data not shown). The four introns within this amplicon are the same size found in *Arabidopsis* and many other dicots such as *Thymus vulgaris* (thyme) and *Dionaea muscipula* (Venus flytrap) (data not shown).

TABLE 4. Act1 *sequencing results in seagrasses and coding sequence similarity to rice*

Species	Total Size (bp)	Nucleotide Similarity to Rice (Exons)	Intron 2 (bp)	Intron 3 (bp)	Family
H. beaudettei	1123	85%	115	137	Cymodoceaceae
C. filiformis	1850	83%	881	97	Cymodoceaceae
H. engelmannii	1108	80%	140	97	Hydrocharitaceae
T. testudinum	1392	81%	119	402	Hydrocharitaceae
R. maritima	1055	80%	94	90	Ruppiaceae

O. sativa sequence used to compare similarity: NM_001057621.1

TABLE 5. Gapdh *sequencing results and coding sequence similarity to rice*

Species	Total Size (bp)	Nucleotide Similarity to Rice (Exons)	Intron 5 (bp)	Intron 6 (bp)	Intron 7 (bp)	Intron 8 (bp)
H. beaudettei	993	85%	110	95	93	89

O. sativa sequence used to compare similarity: EF122472.1

Tissue sample processing and RNA isolation for cDNA

In order to verify putative gene structure and compare exon/intron borders, cDNA was made from RNA extracted from *H. beaudettei* tissues. These tissues were colleted from a different site than tissue samples for genomic DNA extraction due to inaccessibility of the collection site (See Appendix B for coordinates). Assessment of the quality of the total RNA by electrophoresis (Fig. 9) showed that only the rhizome RNA extraction had rRNA bands intact, which indicated that the sample had not degraded. Rhizome RNA was used for making cDNA.

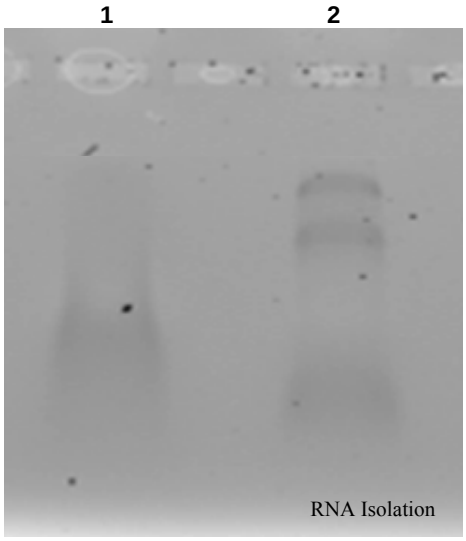

FIG. 9. RNA integrity screening by 1% agarose gel electrophoresis. Each lane was loaded with ~40 ng of total RNA; isolation from *H. beaudettei* blade tissue (lane 1) and *H. beaudettei* rhizome tissue (lane 2).

Cloning and sequencing cDNA

Amplification of *Act1* from cDNA resulted in a band of approximately 900 bp (Fig. 10), near the expected size of 871 bp. A faint band close to 1.5 kb was mostly likely due to non-specific priming and was larger than the 1.1 kb genomic *Act1* amplicon of *H. beaudettei*.

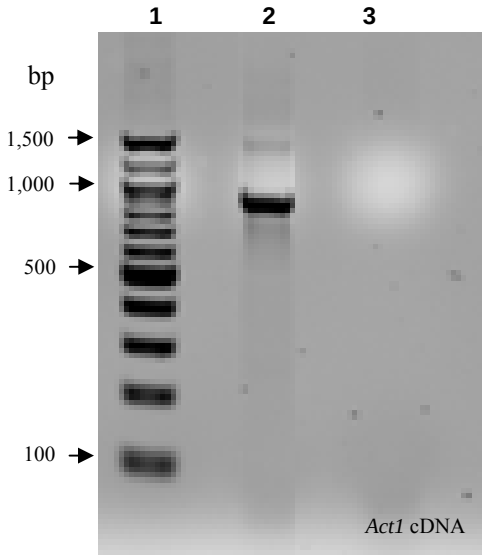

FIG. 10. PCR of *Act1* cDNA from *H. beaudettei* analyzed on 2% agarose gel: 100 bp ladder (lane 1); negative control lacking template DNA (lane 3). Expected size based on genomic amplicon length minus predicted introns = 871 bp.

A similar result was seen for *Gapdh*. Amplification of cDNA for *Gapdh* yielded a major product about 600 bp (Fig. 11). The expected size of *Gapdh* based on genomic amplicon length minus predicted introns is 594 bp. A larger, fainter band above 1kb was most likely due to non-specific priming and the need to optimize the reverse transcriptase reaction.

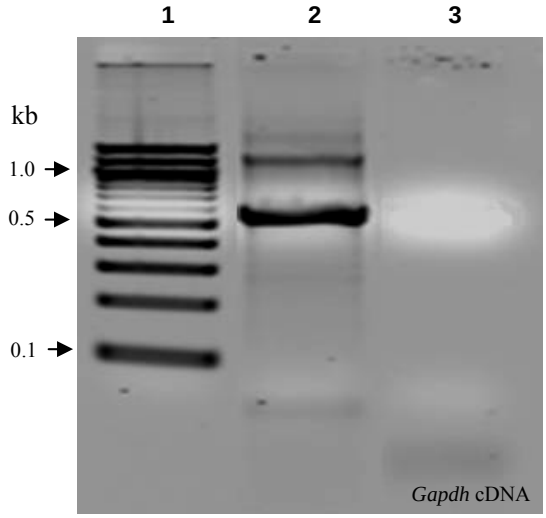

FIG. 11. PCR of *Gapdh* cDNA from *H. beaudettei* analyzed on 2% agarose gel. Size standard: 100 bp ladder (lane 1); negative control lacking template DNA (lane 3). Expected size based on genomic amplicon length minus predicted introns = 594 bp.

Bioinformatics analyses

To assess differences of *H. beaudettei* cDNA sequences from each other and from that of a reference, coding DNA sequences from rice (mRNAs) were used for comparison. *Act1* cDNA sequences had 82-83% similarity to corresponding rice *Act1* coding sequence, while *Gapdh* cDNA sequences had 80-81% similarity (Table 6). These minor variations between clones could indicate allelic differences or expressed isovariants of the cytosolic form of *Gapdh*, prompting comparisons at the protein sequence level.

TABLE 6. *Similarity comparisons of* Act1 *and* Gapdh *cDNA from* H. beaudettei *vs. corresponding sequences in rice*.

Sequence	Total Size (bp)	Similarity to Rice
Act1		
JF775761	871	82%
JF775762	871	82%
JF775763	871	82%
JF775764	871	82%
JF775765	871	82%
JF775766	871	82%
JF775767	871	82%
JF775768	871	83%
Gapdh		
JF775769	594	80%
JF775770	594	81%
JF775771	594	80%
JF775772	594	81%
JF775773	594	81%
JF775774	594	81%
JF775775	594	81%
JF775776	594	81%
JF775777	594	80%

O. sativa mRNA sequences used to compare *Act1* and *Gapdh* similarity were AB047313.1 and GQ848032.1, respectively.

Comparisons of putative protein sequences and properties predicted from cDNA sequences

Genomic and cDNA sequences were used to predict and compare the properties of the partial proteins encoded. Molecular weights of predicted *Act1* partial protein sequences from seagrasses were similar to each other and to the trimmed corresponding sequence in rice (Table 7). The isoelectric points (pI) ranged from 5.26-5.71 (mean 5.45±0.12) compared to rice (pI = 5.45). Since the genetic code is degenerate, codon usage bias can be compared for insight into relatedness between orthologs and paralogs. Codon usage bias was distinctly different in seagrasses vs. rice (13-23% difference). Interestingly, the genomic sequences of *H. engelmannii* and *H. beaudettei* had a higher

mean difference from rice (23.67%) than the other seagrasses; this difference may reflect orthologs with a common gene history before the evolutionary split of these seagrasses from each other.

Surprisingly, coding sequences predicted from cDNA vs. genomic clones from *H. beaudettei* were different as well. This could be due to the differential rates of accumulation of mutations in amplified cDNA (reverse transcriptase followed by Taq amplification) vs. amplified genomic DNA (Taq amplification only). However, frequency calculations and distributions of sequence differences (Figs 12 and 13) argue against this possibility. Because cDNA and genomic clones were derived from rhizome tissues taken from different sites separated by 16.55 km, this could also be a reflection of population-level sequence differences. Alternatively, the unique properties of the cDNAs may reflect their representation of a unique subset of *H. beaudettei* paralogs expressed in the collected sample, which may have been stressed. Interestingly, *H. beaudettei* cDNA sequences and *R. maritima* genomic sequences both had a similar codon difference of 16-17% from rice and had the same theoretical pI (Table 7). This may represent an ortholog reflecting a common gene ancestor before these species diverged.

Molecular weights of predicted *Gapdh* partial protein sequences from seagrasses were similar to each other and to the trimmed corresponding sequence in rice (Table 8). The isoelectric points (pI) ranged from 6.28-8.37 (mean 7.65±0.57) compared to rice (pI = 7.67). Codon usage bias was distinctly different in *H. beaudettei* vs. rice (16-17% difference to rice). Coding sequences predicted from cDNA vs. genomic clones from *H. beaudettei* were also different in their codon usage. Similarly to *Act1* sequence

differences between genomic vs. cDNA, this may be a reflection of population-level sequence differences, or the unique properties of these cDNAs may reflect representation of a unique subset of *H. beaudettei* paralogs.

Referring to Table 8, six of the cDNA sequences had very similar pI values to rice (7.63), while the other three pI values varied substantially (two were basic—8.36 and 8.37; and one was acidic—6.28).

TABLE 7. *Proteomic information on predicted* Act1 *partial protein sequences from seagrasses*.

Sequence	Molecular Weight (g/mol)	Theoretical pI	Length (No. of Amino Acids)	Codon Usage Comparison Mean Difference vs. Rice
H. beaudettei (DNA)				
JF326857	32,168.0	5.72	289	23.81%
JF326858	32,163.9	5.26	289	23.81%
JF326859	32,163.9	5.26	289	23.81%
JF326860	32,216.0	5.58	289	23.67%
H. beaudettei (mRNA)				
JF775761	32,123.8	5.46	289	16.17%
JF775762	32,053.7	5.58	289	16.66%
JF775763	32,077.8	5.58	289	16.38%
JF775764	32,129.7	5.36	289	16.11%
JF775765	32,123.8	5.46	289	16.27%
JF775766	32,192.9	5.58	289	16.13%
JF775767	32,053.7	5.58	289	16.66%
JF775768	32,139.8	5.46	289	17.64%
C. filiformis (DNA)				
JF342678	32,149.8	5.46	289	14.59%
JF342679	32,139.8	5.46	289	14.53%
JF342680	32,107.7	5.46	289	14.50%
JF342681	32,153.8	5.46	289	14.34%
H. engelmannii (DNA)				
JF412039	32,255.0	5.27	289	23.67%
T. testudinum (DNA)				
JF412035	32,212.0	5.35	289	13.47%
JF412036	32,208.1	5.35	289	13.31%
JF412037	32,220.2	5.35	289	13.39%
JF412038	32,208.1	5.35	289	13.44%
R. maritima (DNA)				
JF519825	32,287.0	5.46	289	16.33%
JF519826	32,287.0	5.46	289	16.17%
O. sativa (subsp. Japonica)				
Q10DV7	32,298.0	5.45	289	-

NCBI GenBank accession numbers are given for the seagrass sequences, *O. sativa* sequence from the Swiss-Prot (Uni-Prot) database.

TABLE 8. *Proteomic information on* Gapdh *sequences from* H. beaudettei.

Sequence	Molecular Weight (g/mol)	Theoretical pI	Length (No. of Amino Acids)	Codon Usage Comparison Mean Difference vs. Rice
H. beaudettei (DNA)				
JF14883	21,234.2	7.67	201	20.05%
JF14883 trimmed	20,848.8	7.67	197	20.25%
H. beaudettei (mRNA)				
JF775769	20,822.9	8.36	197	16.44%
JF775770	20,791.8	7.63	197	17.05%
JF775771	20,802.9	8.37	197	16.22%
JF775772	20,803.9	7.63	197	16.72%
JF775773	20,788.8	6.28	197	16.95%
JF775774	20,776.8	7.63	197	16.75%
JF775775	20,791.8	7.63	197	17.05%
JF775776	20,773.8	7.63	197	16.63%
JF775777	20,827.9	7.65	197	16.00%
O. sativa (subsp. Japonica)				
Q0J8A4	20,966.9	6.67	201	-
Q0J8A4 (trimmed)	20,581.5	7.63	197	-

JF775769-JF775777 were amplified from cDNA made from RNA positioned 12 bp inside the genomic amplicon sequence resulting in 4 fewer codons.

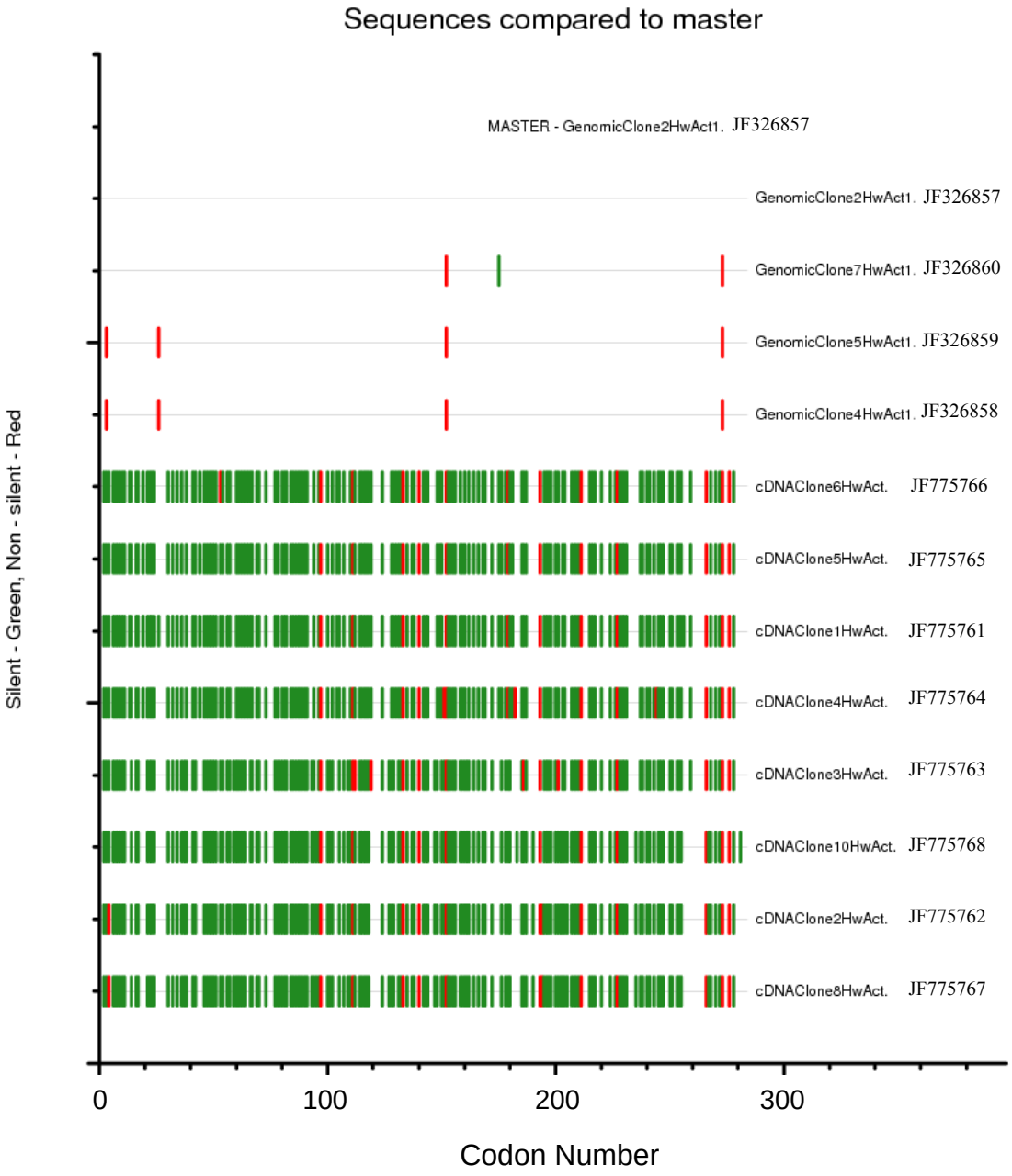

FIG. 12. Silent vs. non-silent mutations among *H. beaudettei Act1* genomic and mRNA sequences. Green hash marks represent silent mutations with red representing non-silent mutations. Sequences are compared with an arbitrary genomic sequence master-JF326857.

The distribution of mutations across the gene and relative to codon position provides insight into sequence differences and suggests they are not a result of random mutational processes during amplification and cloning. *Act1* genomic clones had markedly fewer silent and non-silent differences to the master sequence compared to cDNA clones (Fig. 12). Genomic clones were more like each other than to any of the cDNA sequences, and vice versa. The greatest differences between the genomic clones resulted in 4 non-silent mutations, while the cDNA clones had about 3 times that number of non-silent differences and > 50 silent mutations. When looking at just expressed sequences, there were fewer, but still surprisingly large numbers of differences among the cDNA clones (Fig. 13). The non-silent mutation differences among cDNA sequences ranged from two to five, and the number of silent mutations among cDNA sequences ranged from two to > 40.

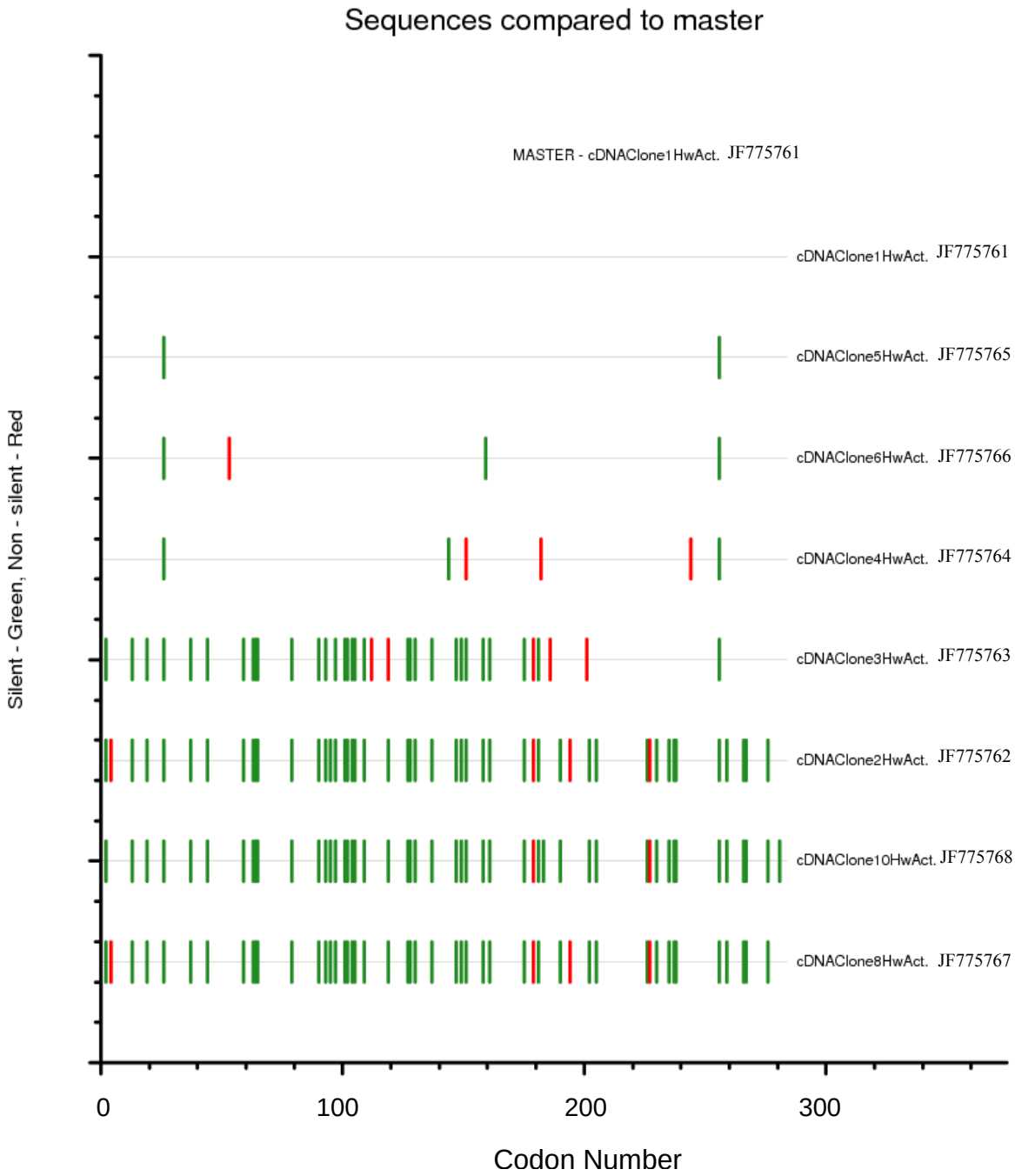

FIG. 13. Silent vs. non-silent mutations among *H. beaudettei Act1* mRNA sequences. Green hash marks represent silent mutations with red representing non-silent mutations. Sequences are compared with an arbitrary mRNA sequence master-JF775761.

Similar comparisons for *Gapdh* genomic and cDNA sequences revealed 22-25 non-silent mutations and > 40 silent mutations (Fig 14). Again, the expressed sequences were more like each other than the genomic sequence with four non-silent and four silent differences at most between each cDNA (Fig 15).

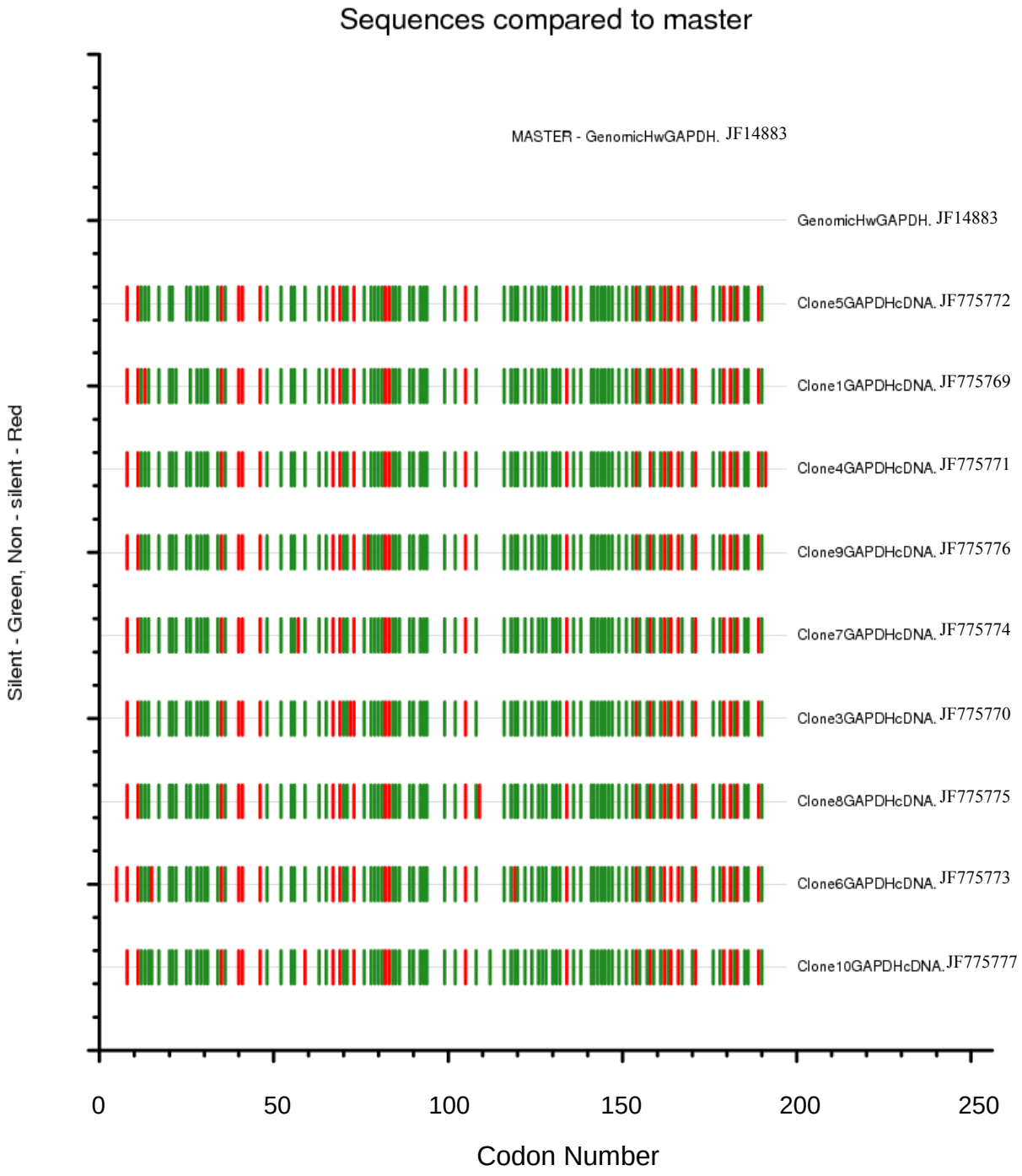

FIG. 14. Silent vs. non-silent mutations among *H. beaudettei* genomic and mRNA *Gapdh* sequences. Green hash marks represent silent mutations with red representing non-silent mutations. Sequences are compared with an arbitrary genomic sequence master-JF14883.

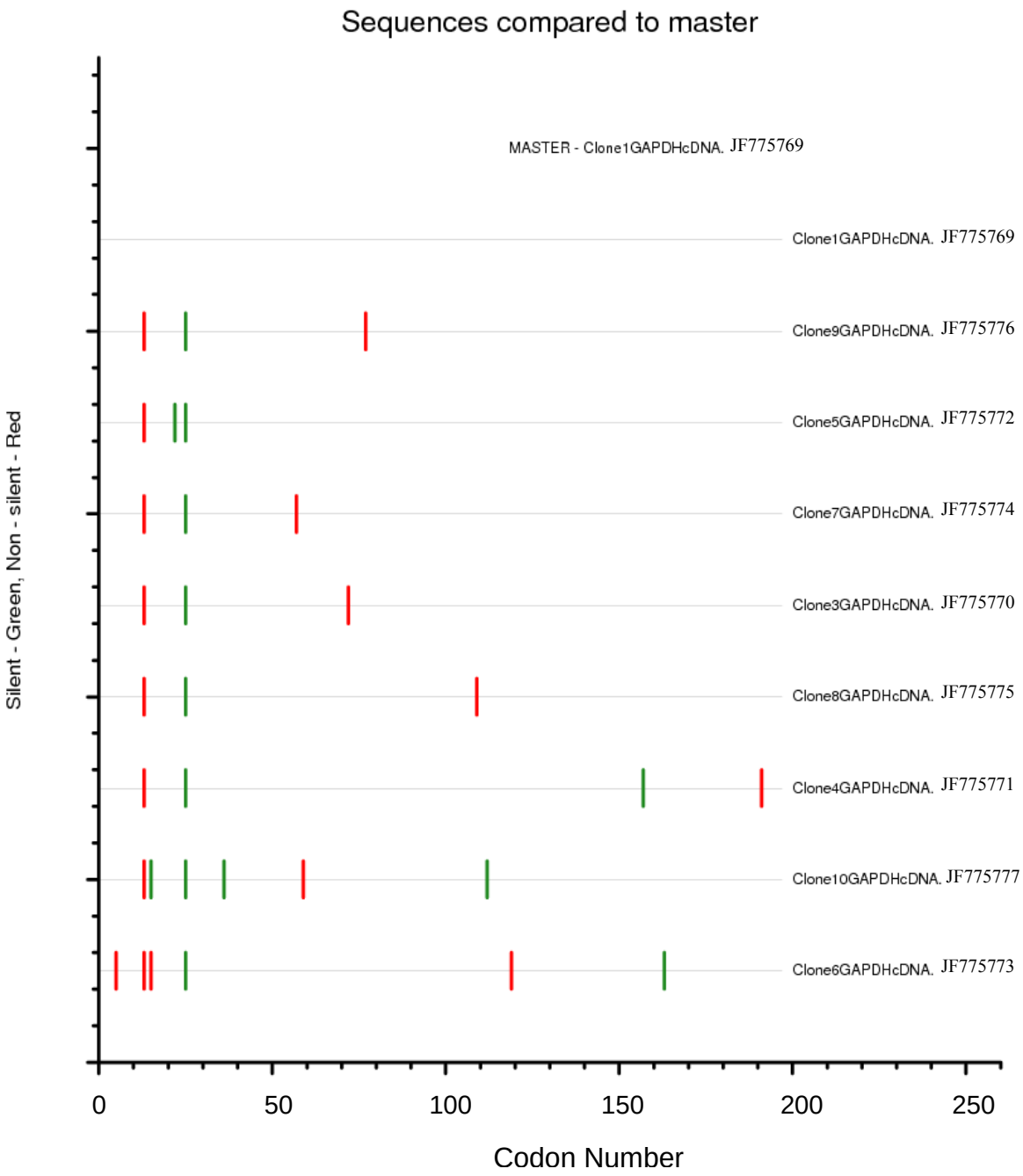

FIG. 15. Silent vs. non-silent mutations among *H. beaudettei Gapdh* mRNA sequences. Green hash marks represent silent mutations with red representing non-silent mutations. Sequences are compared with an arbitrary mRNA sequence master-JF775769.

Constructing phylogenetic trees

To compare actin and GAPDH sequences with each other and with other plants, phylogenetic trees were constructed using Maximum Likelihood. *Act1* genomic sequences from Hydrocharitaceae (*T. testudinum* and *H. engelmannii*) grouped together (Fig. 16). In the Cymodoceaceae family, *H. beaudettei* cDNA sequences and *C. filiformis* genomic sequences grouped together, but the genomic sequences of *H. beaudettei Act1* grouped together with rice rather than with the other seagrasses. *R. maritima* (Ruppiaceae) genomic sequences did not group with any other plant sequences. Interestingly, an actin sequence from *Vallisneria natans*, a freshwater pond weed, did not group with any of the seagrasses either.

H. beaudettei Gapdh cDNA sequences grouped together with other monocot mRNA sequences (Fig. 17), but the *Gapdh* genomic sequence grouped instead with dicot sequences (mostly genomic sequences). Similar results were seen with Neighbor-Joining and Maximum Parsimony-based trees (data not shown), indicating that the expressed cDNA sequences are representative of a different subgroup compared to the genomic sequences of *Gapdh* and *Act1* in *H. beaudettei*.

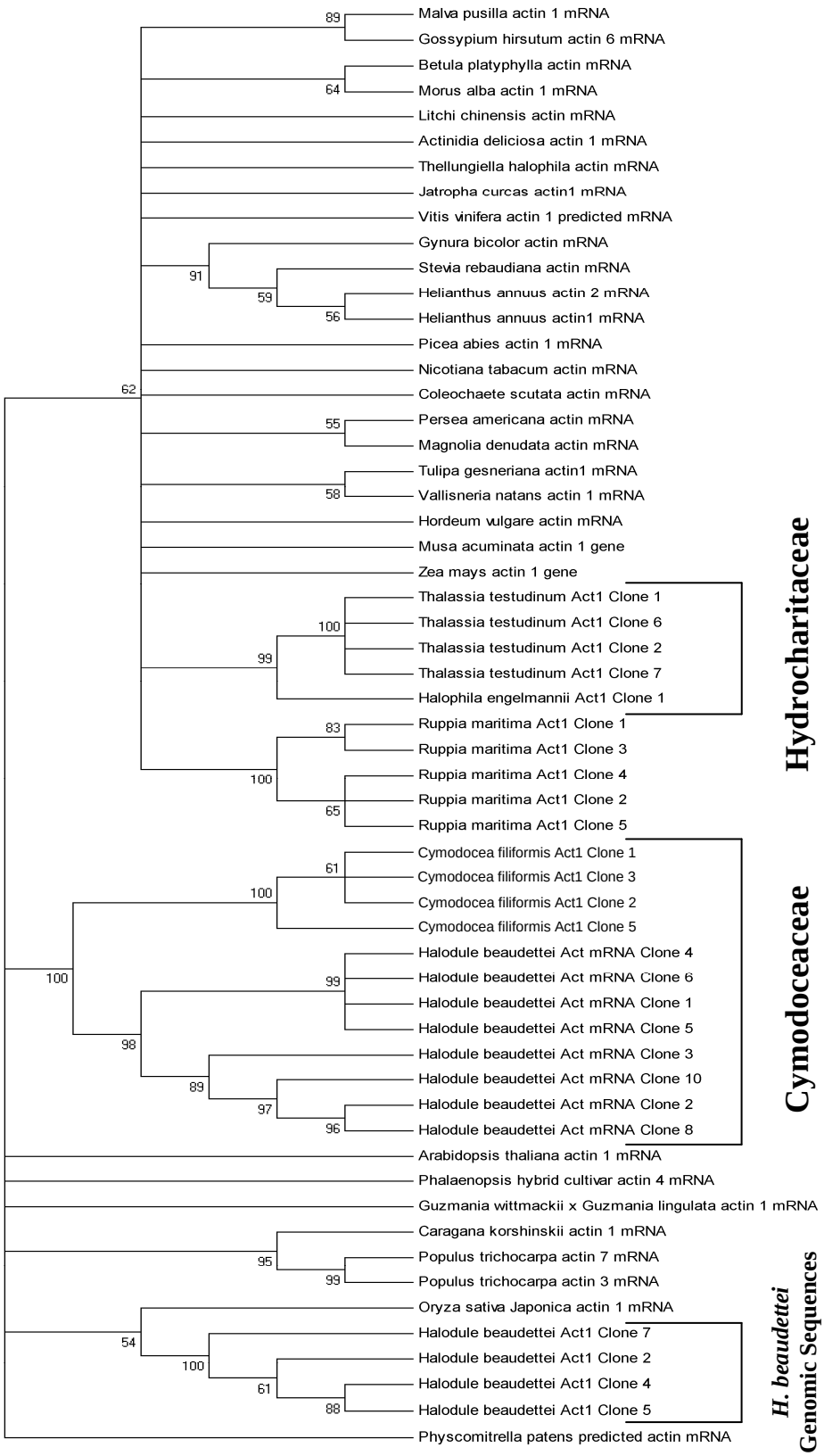

FIG. 16. Maximum Likelihood tree of *Act1* sequences from seagrasses and other plants made with *MEGA*5 using a Kimura 2-parameter model with a Gamma distribution with Invariant sites chosen for rates and patterns of mutations: 1,000 bootstrap replicates.

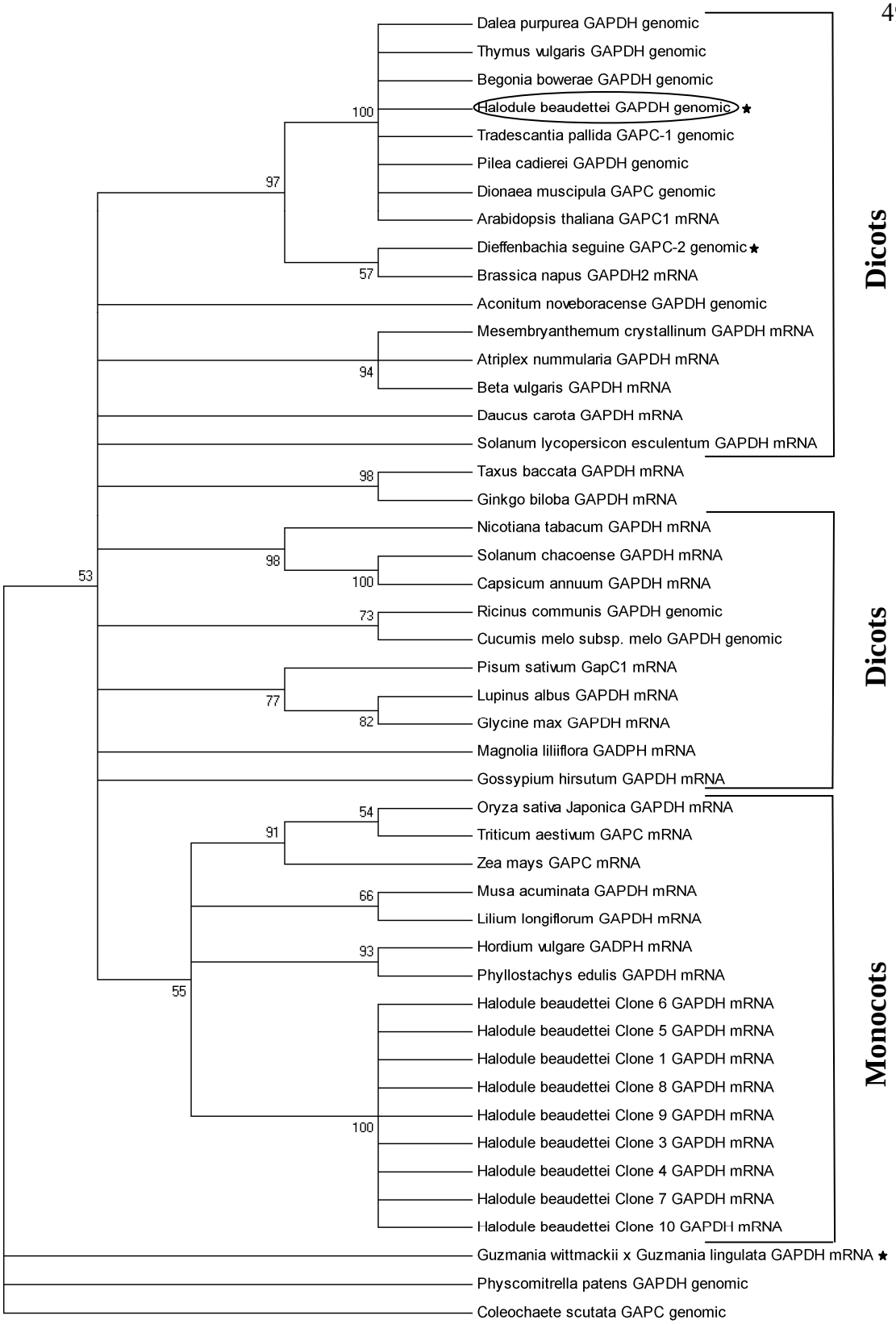

FIG. 17. Maximum Likelihood tree of *Gapdh* sequences from seagrasses and other plants made with *MEGA5* using the General Time Reversal model with a Gamma distribution with Invariant sites chosen for rates and patterns of mutations: 1,000 bootstrap replicates. ★ = monocot

DISCUSSION

In this study, stress-related genes were identified by literature search based on rice. The stress-related candidates identified were *Pal1*, a key regulatory enzyme for synthesis of phenolic compounds, which are involved in secondary metabolism and antimicrobial activity (Harborne, 1977; McMillan *et al*., 1980); *Apx1*, protects against oxidative stress induced by ROS during environmental stresses and/or pathogenic attack (Kotchoni and Gachomo, 2006; Mehdy *et al*., 1996); and *Hb1*, implicated in hypoxic mitochondrial respiration (Igamberdiev *et al*., 2004; Igamberdiev *et* al., 2006; Stoimenova, *et al*., 2007). In addition, the *Act1* and *Gapdh* housekeeping genes were identified to be used as internal controls for future gene-expression assays. *Act1* is a constitutively expressed cytoskeleton component in mature tissues in rice (McElroy *et al*., 1990), whereas *Gapdh* is involved in the sixth step of glycolysis (Cerff, 1982; Russell and Sachs, 1991). For *Gapdh*, PCR nested primers were commercially available from Bio-Rad (Hercules, CA). Primers were developed for all other genes using first amino acid alignment and then nucleotide alignments (Appendix A). Though several primer sets were developed and tested under a variety of PCR conditions, the target stress-related genes could not be amplified (Figs. 3-5). A putative *Hb1* product ~759 bp was cloned and sequenced, but sequence analysis identified this as a bacterial-like NAD/NADP octopine/nopaline dehydrogenase. The DNA source for this sequence could have been from contaminating bacteria, plastids, or mitochondria.

The primers designed had many flaws including non-specific priming and single primer amplification; thus, improvements are needed. When making alignments,

monocots were preferred over dicots in an attempt to locate contiguous conserved residues; this may have posed a problem. The GAPDH genomic sequences grouped together with dicot sequences as opposed to other monocot sequences. A similar result was found with rice *Act1* genomic clones having more similarity with an *Arabidopsis* reproductive actin than with any other rice or plant actins (McElroy *et al.*, 1990). Likewise, some carrot (*Dracus carota*) and maize (*Zea mays*) actins displayed greater similarity to dicots rather than to other monocots (Stranathan *et al*., 1989). It is believed that actin gene duplication took place before the divergence of monocots from dicots (Meagher, 1991), which explains why some monocot actins are more closely related to dicot actins. Incorporating more dicot sequences into alignments may have improved primer efficacy to amplify stress-related genes.

Actin housekeeping genes

In contrast to the stress-related genes, many partial sequences related to the housekeeping gene *Act1* were readily amplified from five seagrass species (*H. beaudettei*, *C. filiformis*, *H. engelmannii*, *T. testudinum*, *Ruppia maritima*). A total of 23 clones (15 genomic clones from all five seagrass species and eight cDNA clones from *H. beaudettei*), corresponding to the middle of exon 2 through exon 4 to the end of the coding sequence including introns, were sequenced. Actin genomic sequence lengths varied (1055-1850 bp) among seagrass species, particularly the Cymodoceaceae and Hydrocharitaceae, due to varied intron lengths (Table 4). For example, *H. beaudettei* had an intron length of 115 bp, but the same intron in *C. filiformis* was 881 bp. The seagrass actin coding regions, while all the same length (871 bp), varied sightly in their degree of

similarity to rice (80-85%). In the poplar tree (*Populus tomentosa*), the eight member actin gene family was found to display extreme conservation in the coding regions and intron/exon borders, but the introns and the 5' UTR lengths varied among members (Zhang *et al*., 2010). Intron lengths also were found to vary in members of the rice and *A. thaliana* actin families (McElroy *et al*., 1990; McDowell *et al*., 1996).

All rice and *A. thaliana* actins investigated have four coding exons and three introns. The gene structure of the actin genomic clones in all seagrass species investigated had similar exon/intron structure, corresponding to exons 2-4 as observed in rice and *A. thaliana* (McElroy *et al*., 1990; McDowell *et al*., 1996). The codon usage and inferred properties of the seagrass actin proteins of the amplified gene segment were investigated. Comparison of codon usage bias can reveal evolutionary information (Murray *et al*., 1989; Kawabe and Miyashita, 2003). Each seagrass species had a unique codon usage compared to rice, and genomic sequences vs. cDNA sequences also varied from each other (Table 7). This may indicate paralogs in *H. beaudettei*. In addition to varying mean codon usage, theoretical pIs displayed slight variations (5.26-5.72), but averaged ~5.45 for all actin sequences (both genomic and cDNAs), which matched that for rice. When comparing silent (synonymous) mutations vs. non-silent (non-synonymous) mutations, genomic actin sequences of *H. beaudettei* are distinct from cDNA sequences (Fig. 12). This could possibly be due to three things: 1) a higher mutation rate in cDNA preparation vs. genomic cDNA amplification; 2) source material population-level DNA sequence differences; or 3) expression-level differences of source material due to environmental conditions. Mutation rates of both reverse-transcriptase

and subsequent Taq polymerase-based PCR of cDNA cannot account for differences seen within the cDNA population. Moreover, the vast majority of sequence differences are silent, meaning the mutations did not accumulate randomly, as would be expected from polymerase errors in amplification. In addition, a number of critically important protein binding sites, including five amino acids involved with ATP binding, nine amino acids interacting with gelsolin, and 11 amino acids interacting with profilin (based on rice), were conserved in both genomic and cDNA clones. Population dynamics seems unlikely to fully explain the numerous mutations, because of the density of the changes in the cDNAs and the proximity of the two sampling sites for genomic vs. cDNA clone source material (Fig. 13). The third possibility, a subset of actins expressed during certain environmental conditions, is most likely the explanation for the differences between the genomic and cDNA clones. It would be insightful to sequence many more genomic actin clones.

Comparisons of the *H. beaudettei* genomic sequences to databases show *H. beaudettei* actins are most closely related to rice *Act1*, which is classified as a "reproductive" actin, though it is expressed constitutively throughout most mature non-reproductive tissues. *A. thaliana Act1*, an ortholog to rice *Act1*, is expressed in mature pollen, pollen tubes, young embryo sac, and organ primordia (McDowell *et al*., 1996; Meagher *et al*., 1999b). Thus, this might explain why this "reproductive-like" actin in *H. beaudettei* was not expressed in rhizomes and therefore not represented in the cDNAs. However, given the large numbers of actin paralogs in some species (Baird and Meagher,

1987; McElroy *el al*., 1990; Meagher, 1991; Meagher and Williamson, 1994), it may also just be fortuitous. The size of the actin family of seagrasses is unknown.

Actins are grouped into two general classes: reproductive and vegetative, with further division into sub-classes in *Arabidopsis* (McDowell *et al*., 1996). Though separation of angiosperm actin genes into two *clearly* demarcated functional groups is not possible, because a large amount of overlap in expression exists between the expression patterns of the different *A. thaliana* genes (McDowell, *et al*., 1996), these two classes do not have equivalent functions (Kandasamy *et al*., 2002). A reproductive actin, *Act1* in *A. thaliana*, engineered to be under the control of the regulatory sequences of a vegetative actin gene, *Act2*, produced dwarfed transgenic plants (Kandasamy *et al*., 2002). This functional non-equivalency can be further understood when protein-protein interactions at the surface of the actin protein are taken into consideration. Many actin binding proteins (ABPs) also occur in gene families (McCurdy *et al*., 2001). Thus, it is not surprising that an actin in *Arabidopsis* from a distantly related actin class could not replace another (Kandasamy *et al*., 2002). Evolutionary studies suggest these two classes diverged very early in vascular plant evolution, around 300-500 million years ago, and that groups of actin and ABPs coevolved (Hightower and Meagher, 1985; McCurdy *et al*., 2001).

Actin and ABP proteins are encoded by large, differentially expressed gene families with individual isoforms displaying biochemically distinct properties (McCurdy *et al*., 2001). It is believed that the complexity found in these gene families has been conserved in vascular plants to maintain a pool of protein isovariants with unique

properties, providing a mechanistic reason for the observed diversity of plant actin functions (McCurdy *et al*., 2001). The "isovariant dynamics" concept is argued to provide robustness to a given biological system, which enables that system to respond to diverse environmental signals (Meagher, *et al*, 1999a). These protein families are believed to have arisen by gene duplication mostly from either unequal crossing-over or genome duplication leading to polyploidization (Ohno, 1970). Retrotransposition of genomic sequences is another source of gene duplication (Moran *el al*., 1999). However, gene duplication through polyploidization likely played a substantial role in plant evolution (Soltis and Soltis, 1995). At least 50% of angiosperm taxa are polyploids (Soltis and Soltis, 1995). Many plants (e.g. *Brassica, Glycine, Gossypium*) that display diploid-like chromosomal behavior have been found to be in fact stabilized or "chomosomally diploidized" polyploids (Wendel, 2000). Polyploidy is common in angiosperms with most species inferred to have experienced at least one polyploidy event in their evolutionary history (Blanc and Wolfe, 2004). *A. thaliana* is thought to have undergone at least two and probably three paleopolyploidy events during its evolutionary history (Adams and Wendel, 2005). It is likely that, over time, some chromosomal segments are saved while others are allowed to be deleted or undergo significant drift. Interestingly, mapping of the chromosomal locations of the actin genes in *A. thaliana* suggests these sequences may have originated from genome polyploidization followed by extensive gene shuffling/reorganization (McKinney and Meagher, 1998). The same study revealed that many actin gene family members mapped closely across the *Arabidopsis* genome with other actins, ABPs, tubulins, and GAPDH genes-both cytosolic

and chloroplastic (McKinney and Meagher, 1998). These groups of tightly linked housekeeping genes may be coordinately regulated under different developmental or environmental conditions.

Gapdh housekeeping genes

A genomic *Gapdh* sequence and nine cDNAs were cloned from *H. beaudettei* (Table 8). The genomic length was 993 bp (the expected size according to Bio-Rad), and the cDNAs were all 594 bp—the expected size minus introns. It appears that multiple isoforms were expressed in the source material and likely exist as multiple genomic copies. The cytosolic form of GAPDH is found in multiple copies in other plants (Russell and Sachs, 1989; Ricard *et al*., 1989; Russell and Sachs, 1991; Pérusse and Schoen, 2004). Most of the predicted pIs for *Gapdh* sequence fragments are similar to the corresponding rice segment, but two expressed *Halodule* gene fragments are more basic and one appears to be more acidic than the corresponding segment in rice (Table 8). Variations in inferred pIs of expressed *Gapdh* fragments in *H. beaudettei* suggest *H. beaudettei* cytosolic forms also occur in a gene family. These differences in pI may play a role in "isovariant dynamics" to give seagrasses the ability to adjust their growth and development according to changing environmental conditions. Meagher *et al*. (1999a) define isovariant dynamics as "the temporal and biochemical expansion of a biological system's responses as a result of the simultaneous expression and interaction of multiple isovariants of a protein." In order to have an "isovariant response," there must be functionally distinct properties (e.g. binding a substrate or cofactor and/or interactions

with other proteins) allowing the isoforms to participate in protein-protein interactions in varying ways (Meagher *et al*., 1999a). This has been hypothesized to lead to more robust and highly buffered responses of cells and to the conservation of gene families (Meagher *et al*., 1999a). Isovariants can differ in their isoelectric points (Meager *et al*, 1999), possibly indicating a dynamic cellular response in rhizome tissues with various cell types and to the surrounding environment.

Comparing genomic and cDNA clone mean codon usage differences to rice *Gapdh* codon bias reveals that the *Halodule* genomic clones are distinctly more different than the cDNAs (Table 8), as was observed in the case of actin. The cDNAs were more similar to each other than to the genomic sequence (Figs 14 and 15). Many silent (synonymous) mutations and non-silent (non-synonymous) mutations were seen in cDNA clones compared to the genomic sequence of *H. beaudettei* (Fig. 14). The same three reasons given for the differences seen in actin sequences apply here as well: 1) a higher mutation rate in cDNA; 2) source material population-level DNA sequence differences; or 3) expression-level differences of source material due to environmental conditions. The third possibility is favored, as explained above (see Fig. 15).

Phylogenetic comparisons demonstrate the *H. beaudettei* genomic GAPDH sequence is most closely related to dicot sequences, in contrast to the cDNA sequences which cluster with monocot GAPDH sequences (Fig. 17). Nucleotide BLAST results from the 993 bp genomic segment, including introns, had dicot GAPDH sequences as top hits with *Thymus vulgaris* (thyme) being the top hit matching 991/993 bp matching with no gaps. The first *A. thaliana* sequence was the ninth on the list with 987/993 bp

matching. The closest monocot sequence was the 72nd down on the list of top 100 hits for this segment, and this was from an mRNA sequence only covering the coding regions (or 61% of the query). Thus, no intron segments from monocots were in the top 100 hits. The primers used for GAPDH amplification were developed based on *A. thaliana* aligned with other plant sequences (Bio-Rad GAPDH PCR Module, Hercules, CA). Thus, the nested PCR might have targeted a more ancient form of the cytosolic *Gapdh* derived before the divergence of monocots from dicots. The genomic sequence may also represent a pseudogene, but all splicing sites at intron/exon borders were present and coding sequences did not diverge from other plant *Gapdh* sequences, unlike what is seen with pseudogenes that diverged from the original coding sequence (McDowell *et al*., 1996; D'Errico *et al*., 2004). It may be that this *Gapdh* is expressed only during certain stressful situations such as hypoxia. Rice was shown to have differential expression patterns of two *Gapdh* genes under anaerobic conditions (Ricard, *et al*, 1989). Similarly, stimulated expression of different *Gapdh* genes was found in *Zea* (corn) and *Arabidopsis* during anoxia, heat shock, and salinity stress (Martinez *et al*., 1989; Russell and Sachs, 1989; Yang *et al*., 1993; Manjunath and Sachs, 1997).

Expression of housekeeping genes

Some isoforms of actin are expressed in response to hormones and pathogen attack. *A. thaliana Act7* is induced by auxin and is important for normal callus formation (Kandasamy *et al*., 2001; Kandasamy *et al*., 2002). Hormones were found to alter expression of specific mRNAs in soybean (Hightower and Meagher, 1985). Biotic stresses, such as pathogen attack, were implicated in the increased expression of an actin

gene (with homology to *Act7* in *Arabidopsis*) in *Malva pusilla* (Jin *et al*., 1999). Taken together, this would seem to suggest that certain GAPDH and actin isovariants could be used in gene-expression assays to assess a stress-related "housekeeping" response.

In conclusion, several actin genes from five seagrass species found along the Texas Gulf Coast were cloned and sequenced. In addition, *Gapdh* from *H. beaudettei* was cloned and sequenced. Comparisons suggest there are multiple classes or isoforms of each housekeeping gene. Gene expression in *H. beaudettei* rhizome tissue showed multiple transcripts can exist at one time for each of these genes, perhaps indicating a dynamic cellular response to environmental conditions, stress from harvesting tissue, or differing isoform expression in each cell type found in rhizome tissue. The ultimate goal is to develop expression assays to monitor seagrass health. Future work should apply Next Generation sequencing to form an (expressed sequence tags) EST database. This would allow a broader, more comprehensive look at gene-expression and perhaps identify more gene families, those members who are correlated with stress, and to identify stress-related gene pathways. This would in turn help monitor seagrass status and give conservationists another tool to manage seagrass beds.

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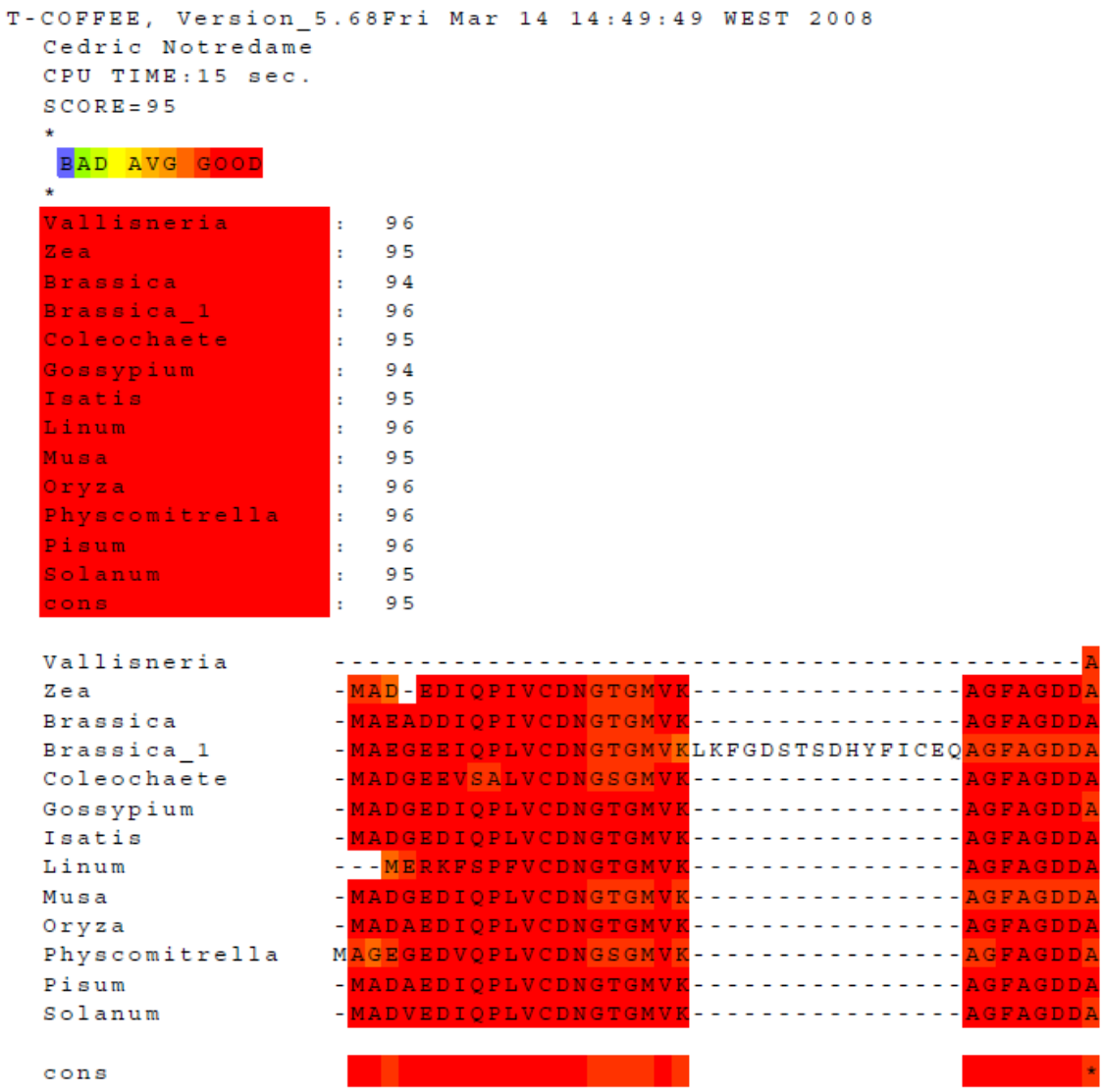
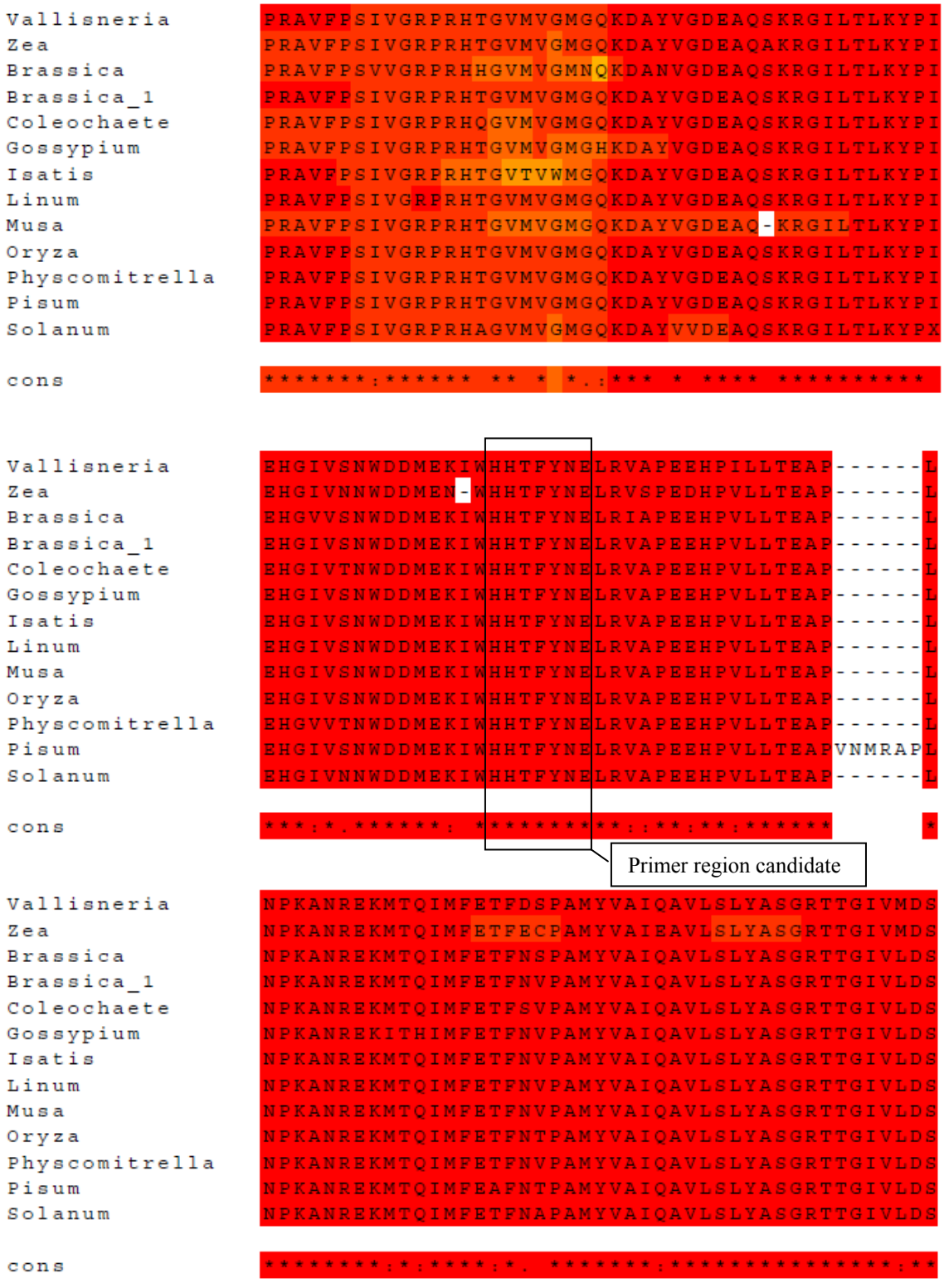
APPENDIX A

Act1 Amino Acid Alignment

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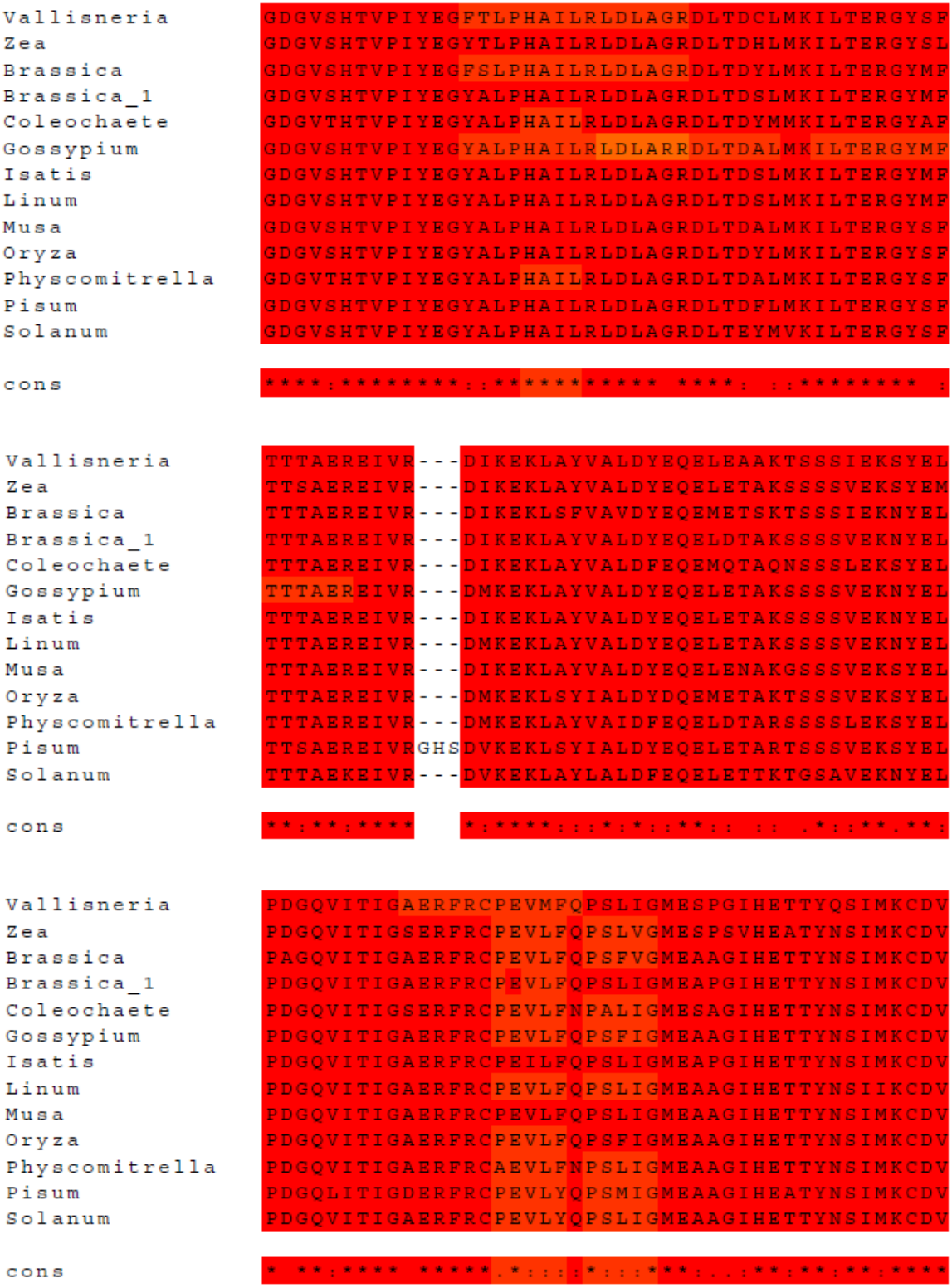
Zea PRAVFPSIVGRPRHTGVMVGMGQKDAYVGDEAQAKRGILTLKYPI
Brassica PRAVFPSVVGRPRHHGVMVGMNQKDANVGDEAQSKRGILTLKYPI
Brassica_1 PRAVFPSIVGRPRHTGVMVGMGQKDAYVGDEAQSKRGILTLKYPI
Coleochaete PRAVFPSIVGRPRHQGVMVGMGQKDAYVGDEAQSKRGILTLKYPI
Gossypium PRAVFPSIVGRPRHTGVMVGMGHKDAYVGDEAQSKRGILTLKYPI
Isatis PRAVFPSIVGRPRHTGVTVWMGQKDAYVGDEAQSKRGILTLKYPI
Linum PRAVFPSIVGRPRHTGVMVGMGQKDAYVGDEAQSKRGILTLKYPI
Musa PRAVFPSIVGRPRHTGVMVGMGQKDAYVGDEAQ-KRGILTLKYPI
Oryza PRAVFPSIVGRPRHTGVMVGMGQKDAYVGDEAQSKRGILTLKYPI
Physcomitrella PRAVFPSIVGRPRHTGVMVGMGQKDAYVGDEAQSKRGILTLKYPI
Pisum PRAVFPSIVGRPRHTGVMVGMGQKDAYVGDEAQSKRGILTLKYPI
Solanum PRAVFPSIVGRPRHAGVMVGMGQKDAYVVDEAQSKRGILTLKYPX
cons
Vallisneria EHGIVSNWDDMEKIWHHTFYNELRVAPEEHPILLTEAP------L
Zea EHGIVNNWDDMEN-WHHTFYNELRVSPEDHPVLLTEAP------L
Brassica EHGVVSNWDDMEKIWHHTFYNELRIAPEEHPVLLTEAP------L
Brassica_1 EHGIVSNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAP------L
Coleochaete EHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAP------L
Gossypium EHGIVSNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAP------L
Isatis EHGIVSNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAP------L
Linum EHGIVSNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAP------L
Musa EHGIVSNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAP------L
Oryza EHGIVSNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAP------L
Physcomitrella EHGVVTNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAP------L
Pisum EHGIVSNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAPVNMRAPL
Solanum EHGIVNNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAP------L
cons
Primer region candidate
Vallisneria NPKANREKMTQIMFETFDSPAMYVAIQAVLSLYASGRTTGIVMDS
Zea NPKANREKMTQIMFETFECPAMYVAIEAVLSLYASGRTTGIVMDS
Brassica NPKANREKMTQIMFETFNSPAMYVAIQAVLSLYASGRTTGIVLDS
Brassica_1 NPKANREKMTQIMFETFNVPAMYVAIQAVLSLYASGRTTGIVLDS
Coleochaete NPKANREKMTQIMFETFSVPAMYVAIQAVLSLYASGRTTGIVLDS
Gossypium NPKANREKITHIMFETFNVPAMYVAIQAVLSLYASGRTTGIVLDS
Isatis NPKANREKMTQIMFETFNVPAMYVAIQAVLSLYASGRTTGIVLDS
Linum NPKANREKMTQIMFETFNVPAMYVAIQAVLSLYASGRTTGIVLDS
Musa NPKANREKMTQIMFETFNVPAMYVAIQAVLSLYASGRTTGIVLDS
Oryza NPKANREKMTQIMFETFNTPAMYVAIQAVLSLYASGRTTGIVLDS
Physcomitrella NPKANREKMTQIMFETFNVPAMYVAIQAVLSLYASGRTTGIVLDS
Pisum NPKANREKMTQIMFEAFNTPAMYVAIQAVLSLYASGRTTGIVLDS
Solanum NPKANREKMTQIMFETFNAPAMYVAIQAVLSLYASGRTTGIVLDS
cons


Vallisneria GDGVSHTVPIYEGFTLPHAILRLDLAGRDLTDCLMKILTERGYSF
Zea GDGVSHTVPIYEGYTLPHAILRLDLAGRDLTDHLMKILTERGYSL
Brassica GDGVSHTVPIYEGFSLPHAILRLDLAGRDLTDYLMKILTERGYMF
Brassica_1 GDGVSHTVPIYEGYALPHAILRLDLAGRDLTDSLMKILTERGYMF
Coleochaete GDGVTHTVPIYEGYALPHAILRLDLAGRDLTDYMMKILTERGYAF
Gossypium GDGVSHTVPIYEGYALPHAILRLDLARRDLTDALMKILTERGYMF
Isatis GDGVSHTVPIYEGYALPHAILRLDLAGRDLTDSLMKILTERGYMF
Linum GDGVSHTVPIYEGYALPHAILRLDLAGRDLTDSLMKILTERGYMF
Musa GDGVSHTVPIYEGYALPHAILRLDLAGRDLTDALMKILTERGYSF
Oryza GDGVSHTVPIYEGYALPHAILRLDLAGRDLTDYLMKILTERGYSF
Physcomitrella GDGVTHTVPIYEGYALPHAILRLDLAGRDLTDALMKILTERGYSF
Pisum GDGVSHTVPIYEGYALPHAILRLDLAGRDLTDFLMKILTERGYSF
Solanum GDGVSHTVPIYEGYALPHAILRLDLAGRDLTEYMVKILTERGYSF
cons
Vallisneria TTTAEREIVR---DIKEKLAYVALDYEQELEAAKTSSSIEKSYEL
Zea TTSAEREIVR---DIKEKLAYVALDYEQELETAKSSSSVEKSYEM
Brassica TTTAEREIVR---DIKEKLSFVAVDYEQEMETSKTSSSIEKNYEL
Brassica_1 TTTAEREIVR---DIKEKLAYVALDYEQELDTAKSSSSVEKNYEL
Coleochaete TTTAEREIVR---DIKEKLAYVALDFEQEMQTAQNSSSLEKSYEL
Gossypium TTTAEREIVR---DMKEKLAYVALDYEQELETAKSSSSVEKNYEL
Isatis TTTAEREIVR---DIKEKLAYVALDYEQELETAKSSSSVEKNYEL
Linum TTTAEREIVR---DMKEKLAYVALDYEQELETAKSSSSVEKNYEL
Musa TTTAEREIVR---DIKEKLAYVALDYEQELENAKGSSSVEKSYEL
Oryza TTTAEREIVR---DMKEKLSYIALDYDQEMETAKTSSSVEKSYEL
Physcomitrella TTTAEREIVR---DMKEKLAYVAIDFEQELDTARSSSSLEKSYEL
Pisum TTSAEREIVRGHSDVKEKLSYIALDYEQELETARTSSSVEKSYEL
Solanum TTTAEKEIVR---DVKEKLAYLALDFEQELETTKTGSAVEKNYEL
cons
Vallisneria PDGQVITIGAERFRCPEVMFQPSLIGMESPGIHETTYQSIMKCDV
Zea PDGQVITIGSERFRCPEVLFQPSLVGMESPSVHEATYNSIMKCDV
Brassica PAGQVITIGAERFRCPEVLFQPSFVGMEAAGIHETTYNSIMKCDV
Brassica_1 PDGQVITIGAERFRCPEVLFQPSLIGMEAPGIHETTYNSIMKCDV
Coleochaete PDGQVITIGSERFRCPEVLFNPALIGMESAGIHETTYNSIMKCDV
Gossypium PDGQVITIGAERFRCPEVLFQPSFIGMEAAGIHETTYNSIMKCDV
Isatis PDGQVITIGAERFRCPEILFQPSLIGMEAPGIHETTYNSIMKCDV
Linum PDGQVITIGAERFRCPEVLFQPSLIGMEAAGIHETTYNSIIKCDV
Musa PDGQVITIGAERFRCPEVLFQPSLIGMEAAGIHETTYNSIMKCDV
Oryza PDGQVITIGAERFRCPEVLFQPSFIGMEAAGIHETTYNSIMKCDV
Physcomitrella PDGQVITIGAERFRCAEVLFNPSLIGMEAAGIHETTYNSIMKCDV
Pisum PDGQLITIGDERFRCPEVLYQPSMIGMEAAGIHEATYNSIMKCDV
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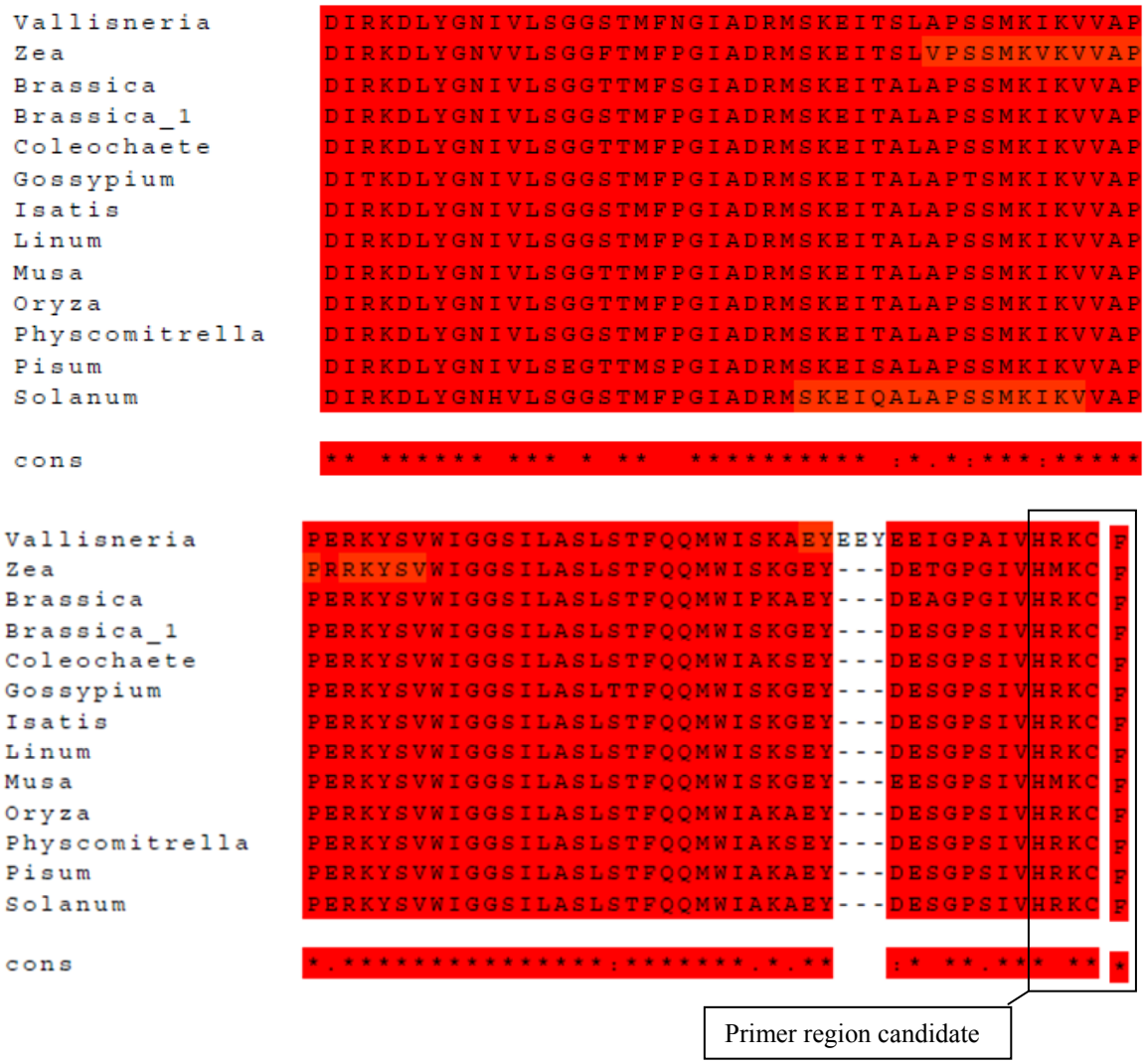
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Brassica_1 DIRKDLYGNIVLSGGSTMFPGIADRMSKEITALAPSSMKIKVVAP
Coleochaete DIRKDLYGNIVLSGGTTMFPGIADRMSKEITALAPSSMKIKVVAP
Gossypium DITKDLYGNIVLSGGSTMFPGIADRMSKEITALAPTSMKIKVVAP
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Pisum DIRKDLYGNIVLSEGTTMSPGIADRMSKEISALAPSSMKIKVVAP
Solanum DIRKDLYGNHVLSGGSTMFPGIADRMSKEIQALAPSSMKIKVVAP
cons
Vallisneria PERKYSVWIGGSILASLSTFQQMWISKAEYEEYEEIGPAIVHRKC F
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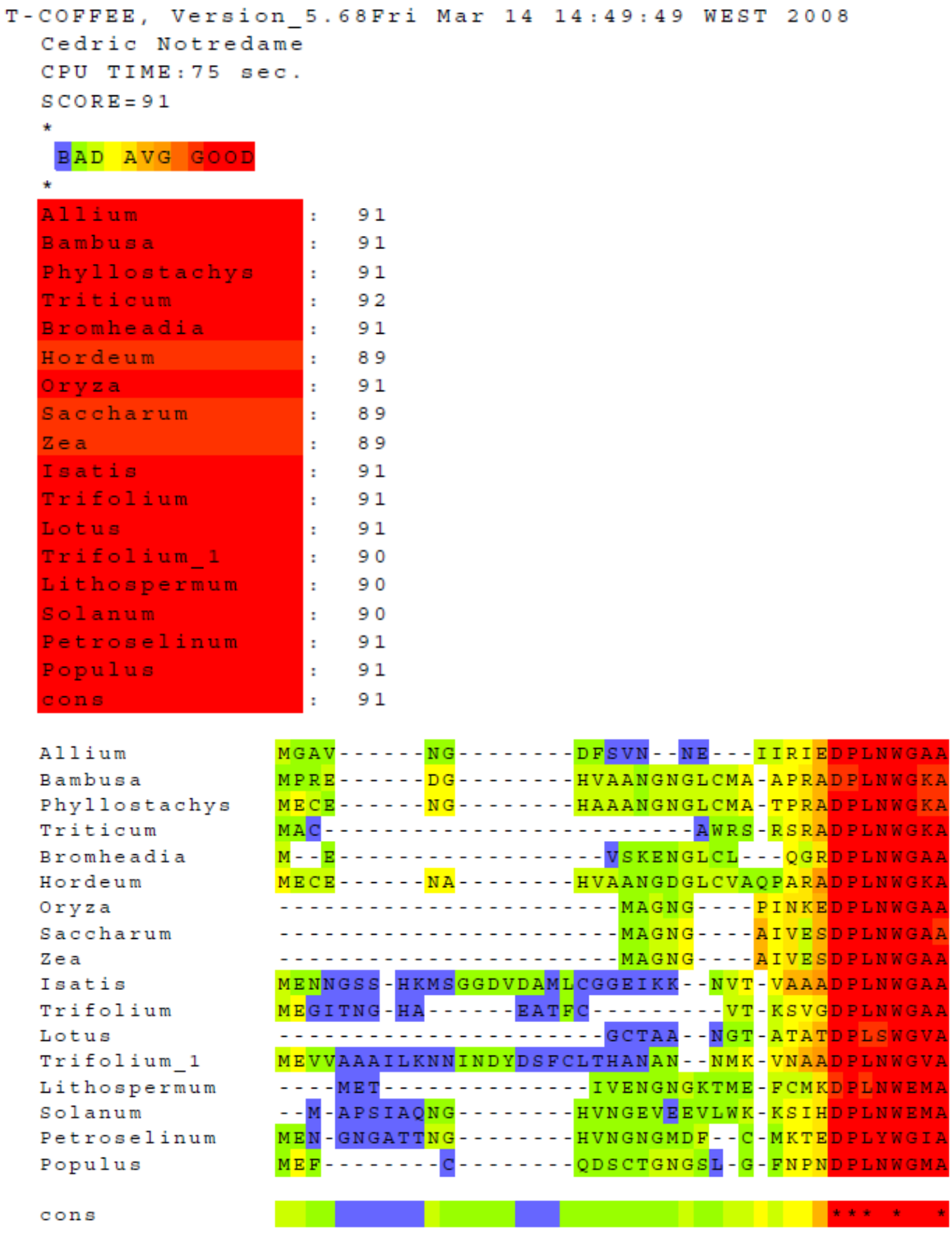
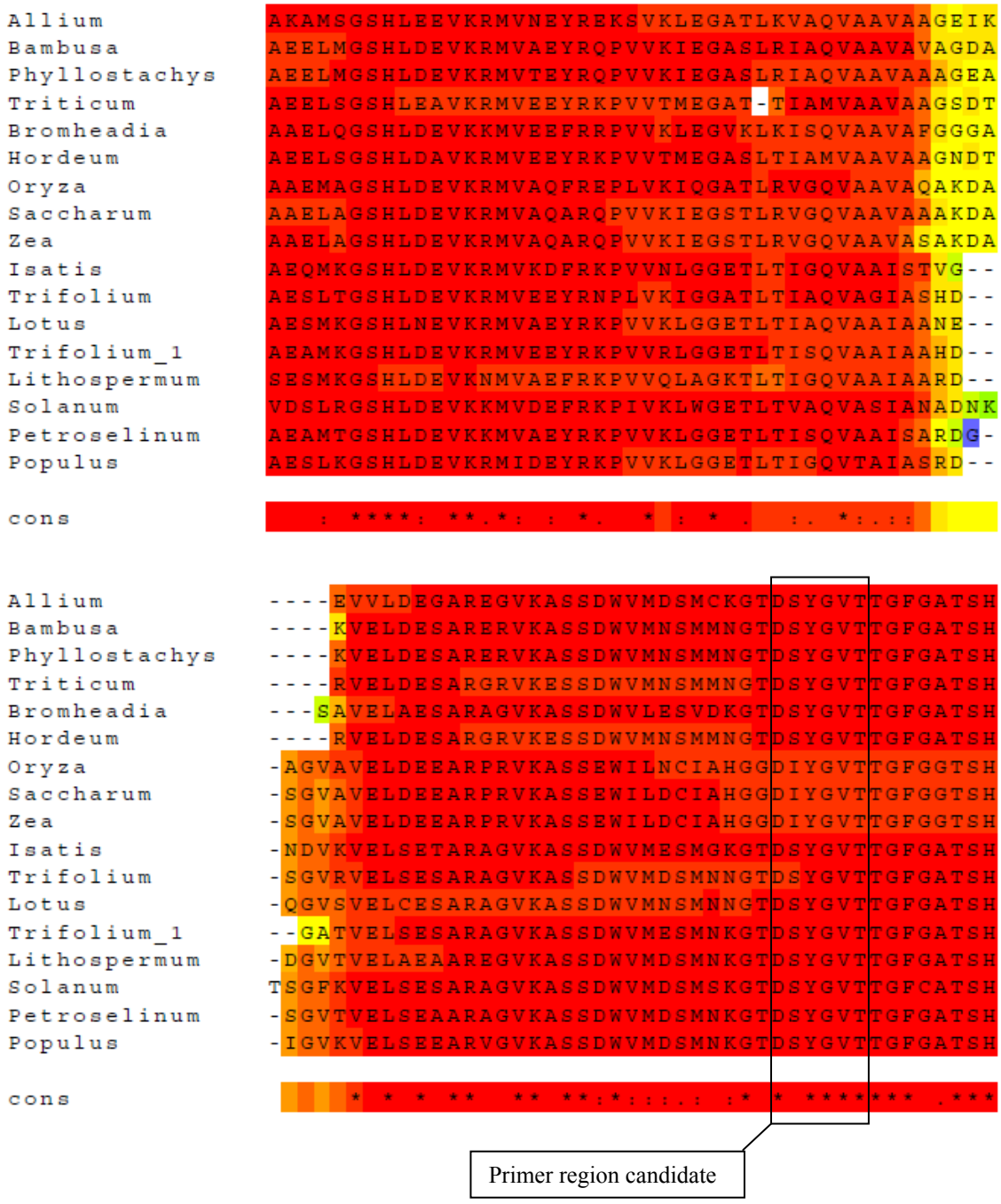
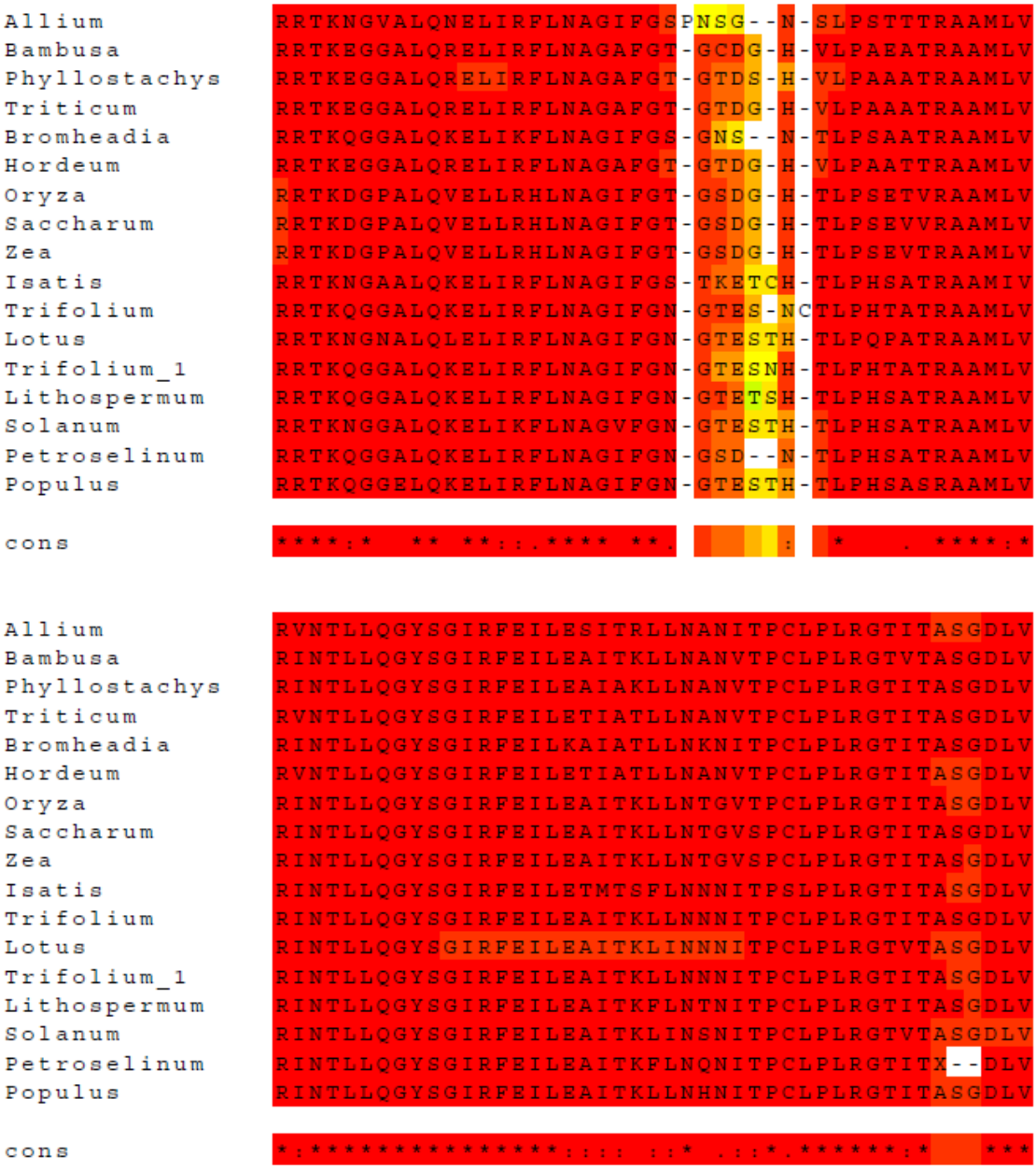
cons
Primer region candidate

Pal1 Amino Acid Alignment




Allium
Bambusa
Phyllostachys
Triticum
Bromheadia
Hordeum
Oryza
Saccharum
Zea
Isatis
Trifolium
Lotus
Trifolium_1
Lithospermum
Solanum
Petroselinum
Populus
cons
Primer region candidate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Phyllostachys RRTKEGGALQRELIRFLNAGAFGT-GTDS-H-VLPAAATRAAMLV
Triticum RRTKEGGALQRELIRFLNAGAFGT-GTDG-H-VLPAAATRAAMLV
Bromheadia RRTKQGGALQKELIKFLNAGIFGS-GNS--N-TLPSAATRAAMLV
Hordeum RRTKEGGALQRELIRFLNAGAFGT-GTDG-H-VLPAATTRAAMLV
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Lithospermum RRTKQGGALQKELIRFLNAGIFGN-GTETSH-TLPHSATRAAMLV
Solanum RRTKNGGALQKELIKFLNAGVFGN-GTESTH-TLPHSATRAAMLV
Petroselinum RRTKQGGALQKELIRFLNAGIFGN-GSD--N-TLPHSATRAAMLV
Populus RRTKQGGELQKELIRFLNAGIFGN-GTESTH-TLPHSASRAAMLV
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Bromheadia RINTLLQGYSGIRFEILKAIATLLNKNITPCLPLRGTITASGDLV
Hordeum RVNTLLQGYSGIRFEILETIATLLNANVTPCLPLRGTITASGDLV
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Trifolium RINTLLQGYSGIRFEILEAITKLLNNNITPCLPLRGTITASGDLV
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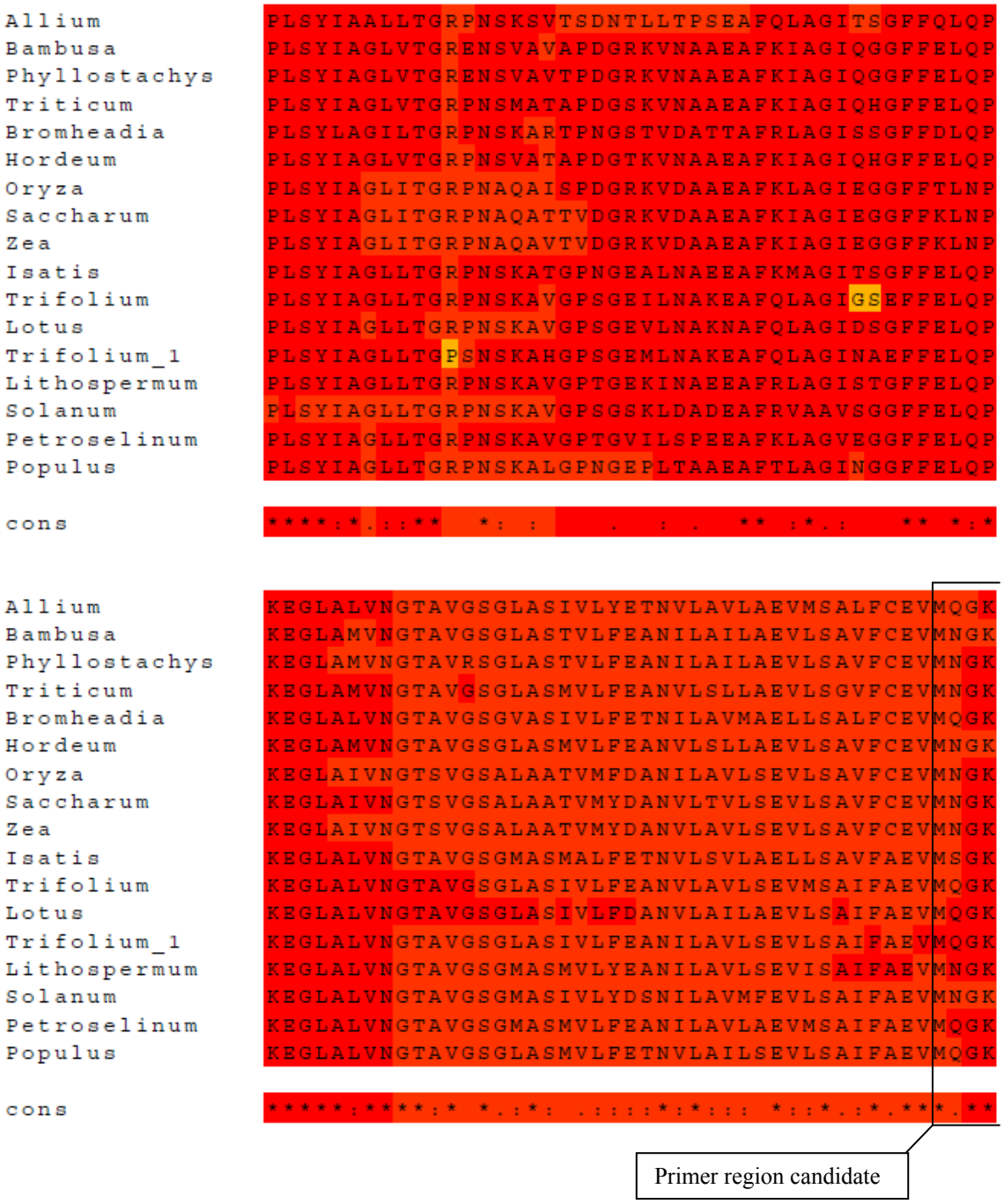
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Solanum PLSYIAGLLTGRPNSKAVGPSGSKLDADEAFRVAAVSGGFFELQP
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Populus PLSYIAGLLTGRPNSKALGPNGEPLTAAEAFTLAGINGGFFELQP
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cons
Primer region candidate


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Oryza PEYTDHLTHKLKHHPGSIEAAAIMEHILAGSSFMSHAKKVNEMDP
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Zea PEYTDHLTHKLKHHPGSIEAAAIMEHILDGSSFMKQAKKVNELDP
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Phyllostachys LMKPKQDRYALRTSPQWLGPQIEVIRAATKSIEREINSVNDNPLI
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Bromheadia LQKPKQDRYALRTSPQWLGPQIEVIRAATKSIEREINSVNDNPLI
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Trifolium LQKPKQDRYALRTSPQWLGPLIEVIRFSTKSIEREINSVNDNPLI
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Trifolium_1 LQKPKQDRYALRTSPQWLGPLIEVIRFSTKSIEREINSVNDNPLI
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Solanum LQKPKQDRYALRTSPQWLGPQIEVIRAATKMIEREINSVNDNPLI
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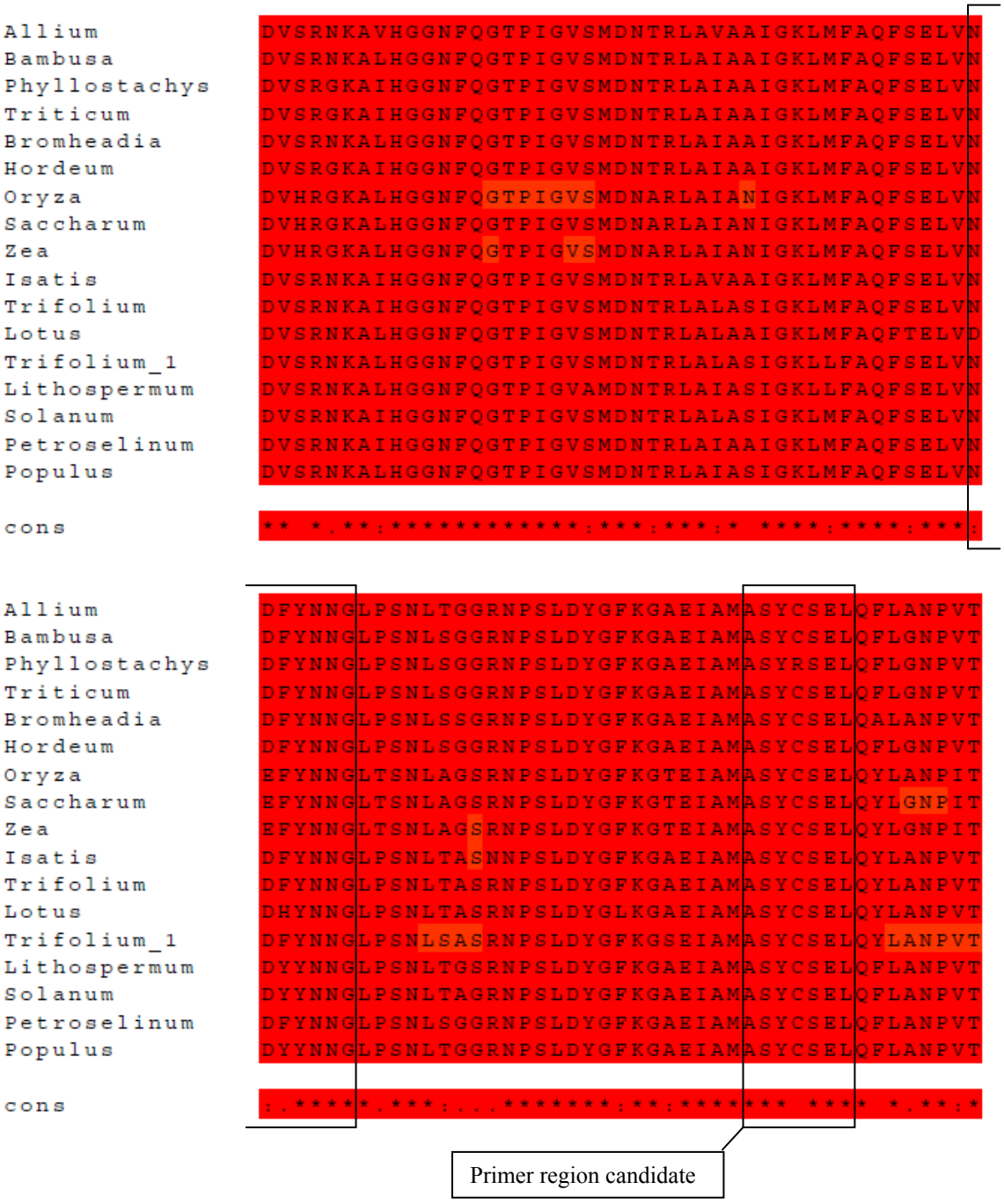
cons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Bromheadia DVSRNKALHGGNFQGTPIGVSMDNTRLAIAAIGKLMFAQFSELVN
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Populus DVSRNKALHGGNFQGTPIGVSMDNTRLAIASIGKLMFAQFSELVN
cons
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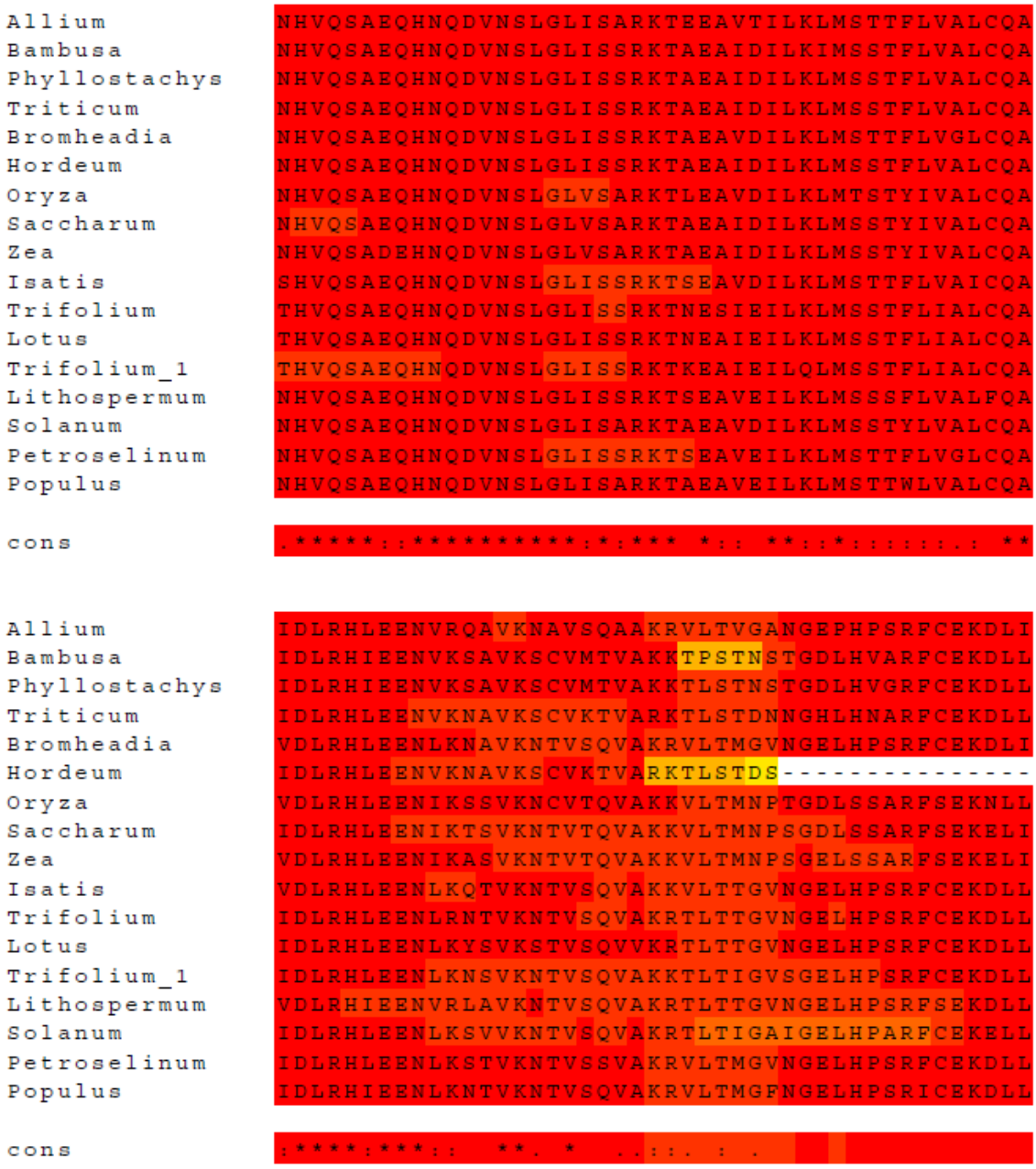
cons
Primer region candidate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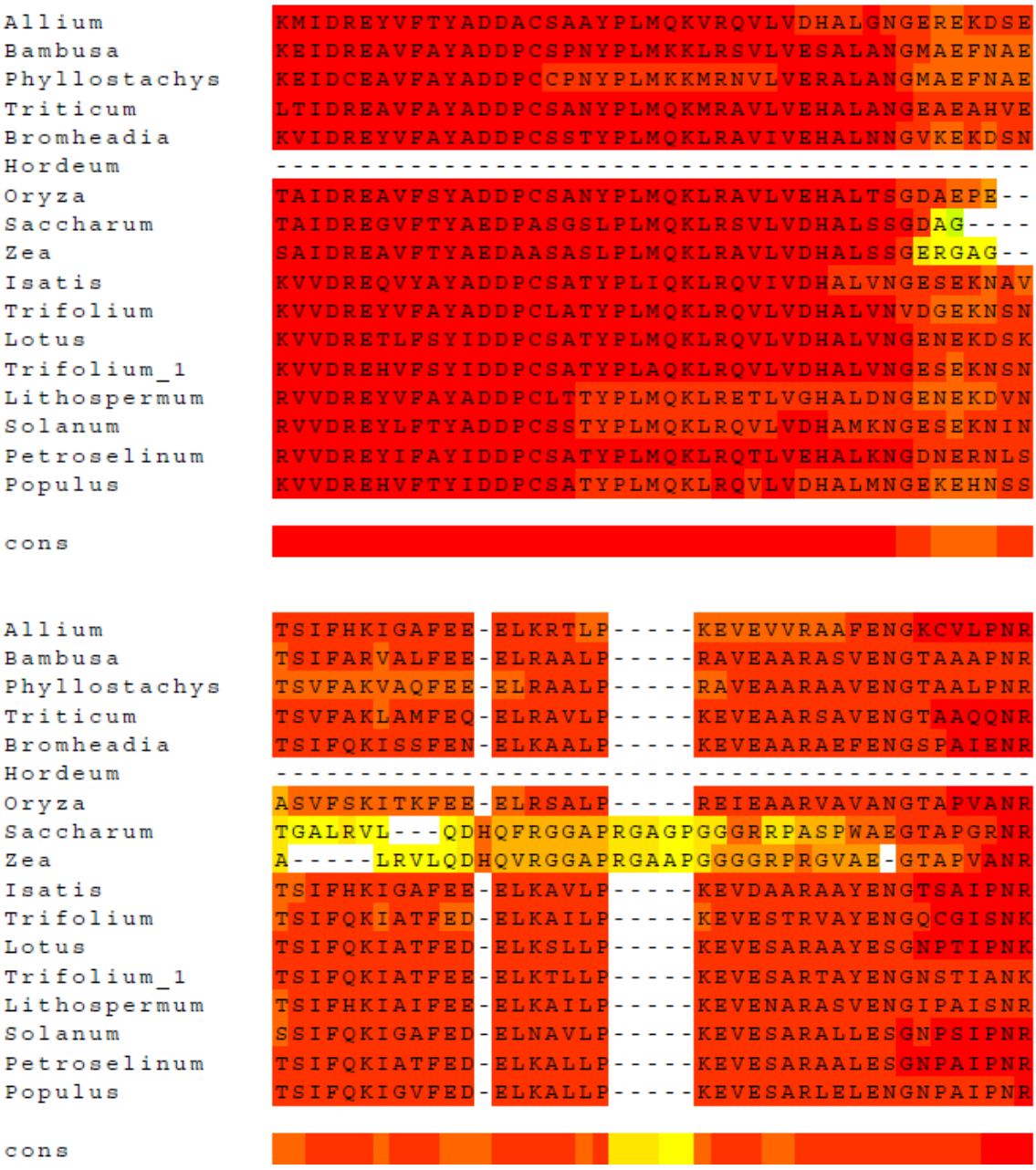
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Hordeum ----------
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Saccharum TGALRVL---QDHQFRGGAPRGAGPGGGRRPASPWAEGTAPGRNR
Zea A-----LRVLQDHQVRGGAPRGAAPGGGGRPRGVAE-GTAPVANR
Isatis TSIFHKIGAFEE-ELKAVLP-----KEVDAARAAYENGTSAIPNR
Trifolium TSIFQKIATFED-ELKAILP-----KEVESTRVAYENGQCGISNK
Lotus TSIFQKIATFED-ELKSLLP-----KEVESARAAYESGNPTIPNK
Trifolium_1 TSIFQKIATFEE-ELKTLLP-----KEVESARTAYENGNSTIANK
Lithospermum TSIFHKIAIFEE-ELKAILP-----KEVENARASVENGIPAISNR
Solanum SSIFQKIGAFED-ELNAVLP-----KEVESARALLESGNPSIPNR
Petroselinum TSIFQKIATFED-ELKALLP-----KEVESARAALESGNPAIPNR
Populus TSIFQKIGVFED-ELKALLP-----KEVESARLELENGNPAIPNR
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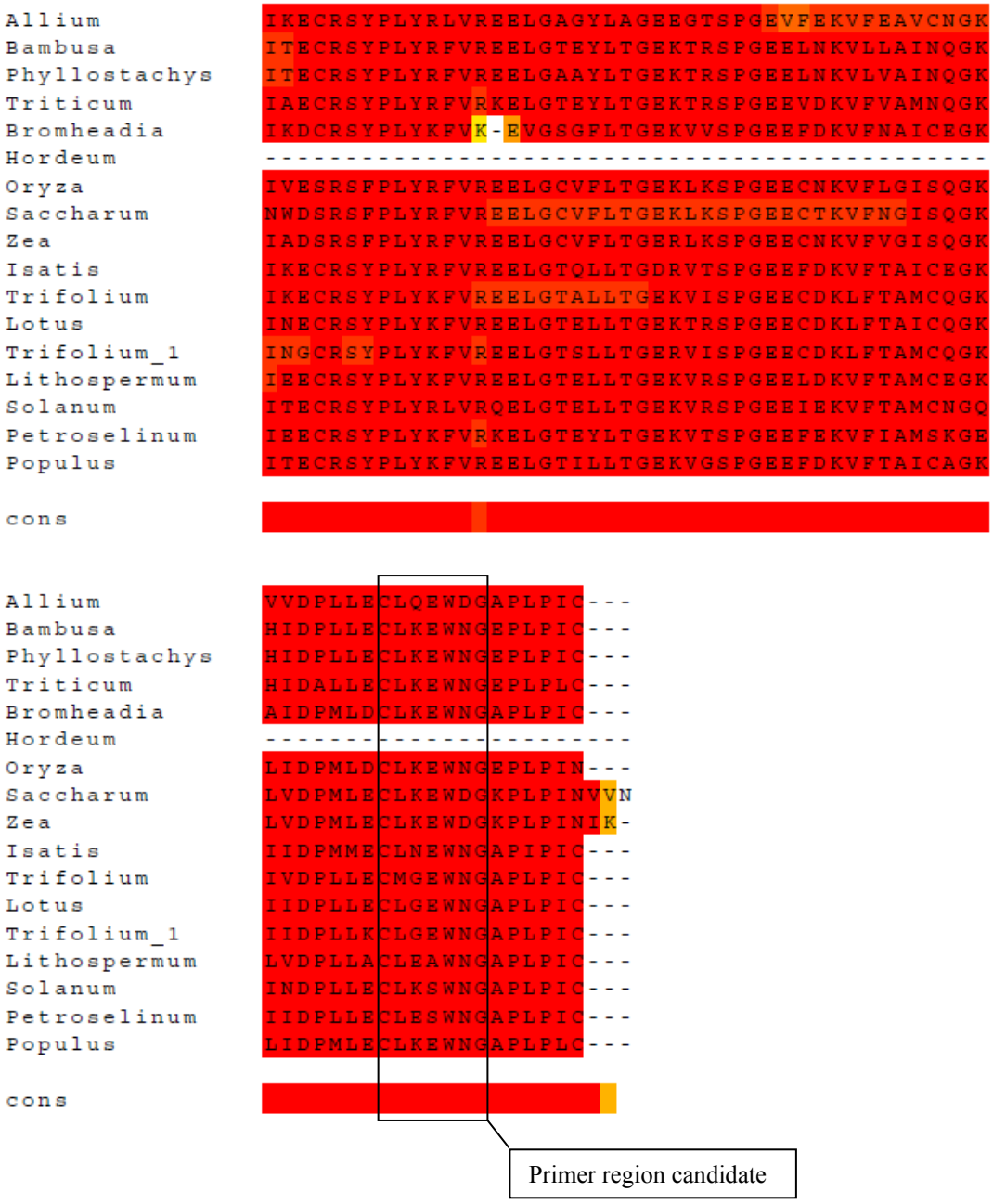
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Phyllostachys ITECRSYPLYRFVREELGAAYLTGEKTRSPGEELNKVLVAINQGK
Triticum IAECRSYPLYRFVRKELGTEYLTGEKTRSPGEEVDKVFVAMNQGK
Bromheadia IKDCRSYPLYKFVK-EVGSGFLTGEKVVSPGEEFDKVFNAICEGK
Hordeum ----------
Oryza IVESRSFPLYRFVREELGCVFLTGEKLKSPGEECNKVFLGISQGK
Saccharum NWDSRSFPLYRFVREELGCVFLTGEKLKSPGEECTKVFNGISQGK
Zea IADSRSFPLYRFVREELGCVFLTGERLKSPGEECNKVFVGISQGK
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Trifolium IKECRSYPLYKFVREELGTALLTGEKVISPGEECDKLFTAMCQGK
Lotus INECRSYPLYKFVREELGTELLTGEKTRSPGEECDKLFTAICQGK
Trifolium_1 INGCRSYPLYKFVREELGTSLLTGERVISPGEECDKLFTAMCQGK
Lithospermum IEECRSYPLYKFVREELGTELLTGEKVRSPGEELDKVFTAMCEGK
Solanum ITECRSYPLYRLVRQELGTELLTGEKVRSPGEEIEKVFTAMCNGQ
Petroselinum IEECRSYPLYKFVRKELGTEYLTGEKVTSPGEEFEKVFIAMSKGE
Populus ITECRSYPLYKFVREELGTILLTGEKVGSPGEEFDKVFTAICAGK
cons
Allium VVDPLLECLQEWDGAPLPIC---
Bambusa HIDPLLECLKEWNGEPLPIC---
Phyllostachys HIDPLLECLKEWNGEPLPIC---
Triticum HIDALLECLKEWNGEPLPLC---
Bromheadia AIDPMLDCLKEWNGAPLPIC---
Hordeum ----------
Oryza LIDPMLDCLKEWNGEPLPIN---
Saccharum LVDPMLECLKEWDGKPLPINVVN
Zea LVDPMLECLKEWDGKPLPINIK-
Isatis IIDPMMECLNEWNGAPIPIC---
Trifolium IVDPLLECMGEWNGAPLPIC---
Lotus IIDPLLECLGEWNGAPLPIC---
Trifolium_1 IIDPLLKCLGEWNGAPLPIC---
Lithospermum LVDPLLACLEAWNGAPLPIC---
Solanum INDPLLECLKSWNGAPLPIC---
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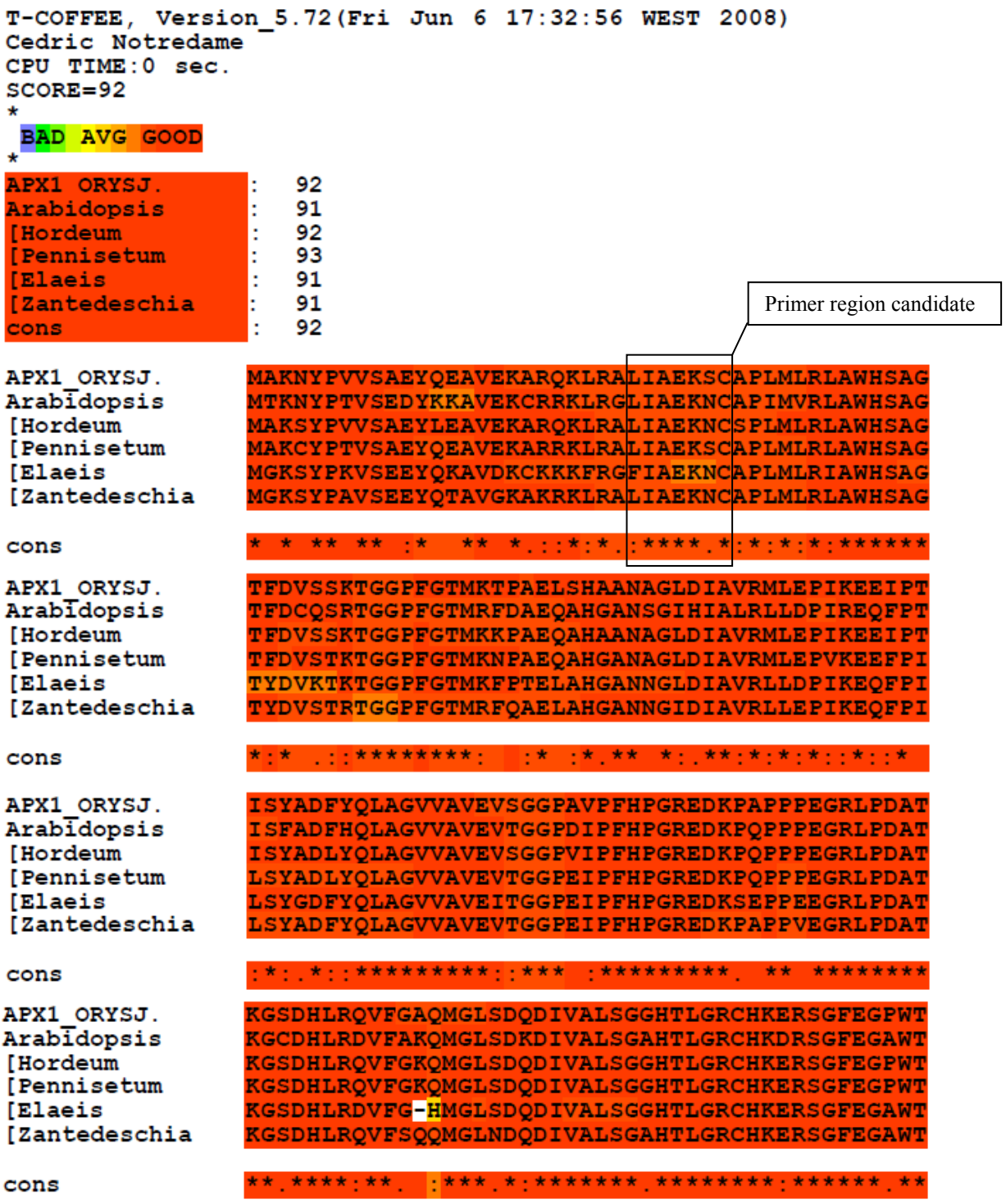
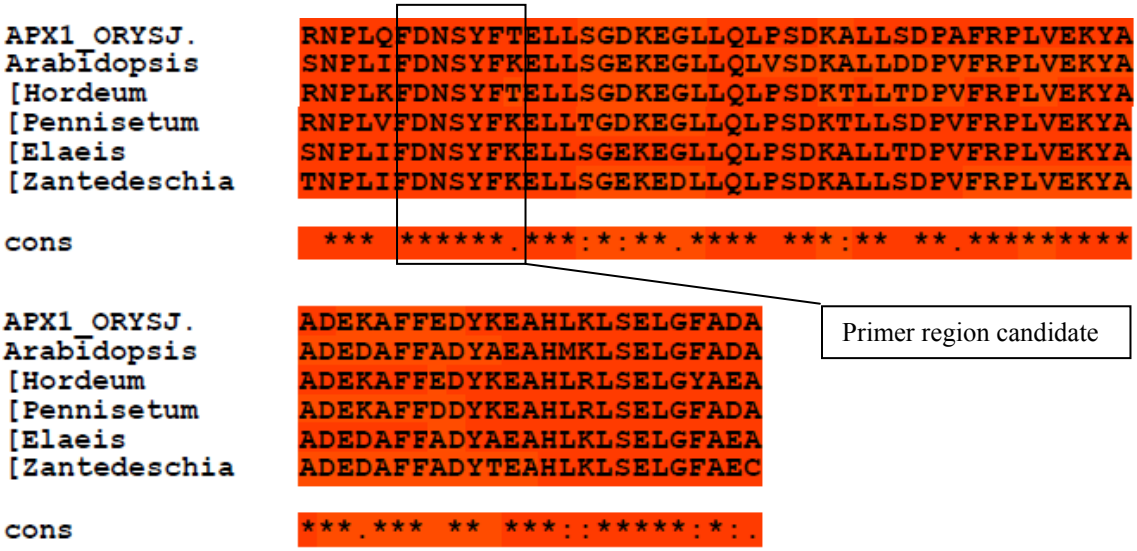
Populus LIDPMLECLKEWNGAPLPLC---
cons
Primer region candidate

Apx1 Amino Acid Alignment

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cons
Primer region candidate

Hb1 Amino Acid Alignment

Act1 Nucleotide Alignment

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SCORE=30
*
 BAD AVG GOOD
*
Elaeis          :  26
Hordeum         :  31
Oryza           :  32
S.vulgare.      :  32
Zea             :  31
Setaria         :  28
Populus         :  28
cons            :  30

Elaeis          ATGGCAGATGCCGAGGATATCCAACCTCTTGTCTGTGACAACGGTACC
Hordeum         ATGGCTGACGCCGAGGACATCCAGCCCCTCGTCTGCGACAATGGTACC
Oryza           ATGGCTGACGCCGAGGATATCCAGCCCCTCGTCTGCGATAATGGAACT
S.vulgare.      ATGGCTGACGCCGAGGATATCCAGCCCCTCGTCTGCGACAATGGAACC
Zea             ATGGCCGATGCCGAGGATATCCAGCCCCTCGTCTGCGACAACGGAACT
Setaria         ATGGCGGACGGTGAAGATATCCAGCCCCTTGTCTGCGACAATGGCACC
Populus         ATGGCAGACGCAGAGGATATTCAGCCACTCGTTTGCGACAATGGAACT

cons            ***** ** *  ** ** ** ** ** ** ** ** ** ** ** **


Elaeis          GGAATGGTCAAGGCTGGATTTGCTGGTGATGATGCACCGAGGGCAGTA
Hordeum         GGTATGGTCAAGGCTGGGTTCGCTGGAGATGATGCTCCCAGGGCTGTC
Oryza           GGTATGGTCAAGGCTGGGTTCGCCGGAGATGATGCGCCCAGGGCTGTC
S.vulgare.      GGTATGGTCAAGGCTGGGTTCGCTGGAGATGACGCCCCAGGGCCGTC
Zea             GGCATGGTCAAGGCTGGGTTCGCTGGCGACGACGCCCCGAGGGCCGTC
Setaria         GGCATGGTCAAGGCCGGTTTCGCAGGGGATGATGCGCCGAGGGCTGTC
Populus         GGAATGGTCAAGGCTGGATTTGCTGGAGATGATGCTCCAAGAGCTGTC

cons            ** ********** ** ** ** ** ** ** ** ** ** ** **

Elaeis          TTTCCCAGTATTGTAGGCCGACCTCGTCACACGGGTGTCATGGTTGGC
Hordeum         TTCCCCAGTATCGTGGGCCGCCCACGCCACACCGGTGTCATGGTCGGG
Oryza           TTCCCCAGCATTGTCGGCCGCCCTCGCCACACCGGTGTCATGGTCGGA
S.vulgare.      TTCCCCAGCATTGTCGGCCGGCCGCGCCACACCGGTGTCATGGTCGGG
Zea             TTCCCCAGCATCGTTGGGCGCCCGCGCCACACCGGTGTGATGGTGGGG
Setaria         TTCCCGAGTATTGTTGGACGCCCGCGCCACACCGGCGTGATGGTGGGA
Populus         TTTCCCAGTATTGTTGGTCGTCCTCGTCACACTGGTGTGATGGTTGGC

cons            ** ** ** ** ** ** ** ** ** ***** ** ** ***** **
```

```
Elaeis          ATGGGCCAAAAGGATGCATATGTTGGTGATGAAGCCCAGTCTAAAAGA
Hordeum         ATGGGGCAGAAGGACGCCTACGTCGGTGACGAGGCGCAGTCCAAGAGG
Oryza           ATGGGCCAGAAGGACGCCTACGTCGGCGACGAGGCGCAGTCCAAGAGG
S.vulgare.      ATGGGGCAGAAGGACGCCTACGTTGGTGACGAGGCGCAGTCCAAGAGG
Zea             ATGGGGCAGAAGGATGCCTACGTCGGCGACGAGGCGCAGTCCAAGAGG
Setaria         ATGGGGCAGAAGGACGCCTATGTTGGCGATGAGGCCCAGTCCAAGAGG
Populus         ATGGGCCAGAAAGATGCATATGTCGGTGATGAGGCTCAGTCCAAGAGA

cons            ***** ** ** ** ** ** ** ** ** ** ** ***** ** **

Elaeis          GGTATCCTCACCTTGAAATACCCCATCGAGCATGGCATTGTTAATAAC
Hordeum         GGTATCTTGACTCTCAAGTACCCCATTGAGCACGGTATCGTCAGCAAC
Oryza           GGTATCTTGACCCTCAAGTACCCCATCGAGCATGGTATCGTCAGCAAC
S.vulgare.      GGTATCCTGACCCTCAAGTACCCCATCGAGCACGGAATCGTCAGCAAC
Zea             GGTATCCTGACCCTCAAGTACCCCATCGAGCACGGAATCGTCAGCAAC
Setaria         GGTATCCTCACCCTGAAGTACCCAATCGAGCACGGTATCGTCAGCAAC
Populus         GGTATCTTAACTTTGAAATACCCAATTGAGCATGGTATTGTGAGCAAT

cons            ****** * **  * ** ***** ** ***** ** ** ** *  **

Elaeis          TGGGATGATATGGAGAAGATCTGGCATCACACTTTCTACAATGAGCTC
Hordeum         TGGGACGATATGGAGAAGATCTGGCATCACACCTTCTACAACGAGCTC
Oryza           TGGGATGATATGGAGAAGATCTGGCATCACACCTTCTACAACGAGCTC
S.vulgare.      TGGGACGATATGGAGAAGATCTGGCATCACACCTTCTACAACGAGCTC
Zea             TGGGACGACATGGAGAAGATCTGGCATCACACCTTCTACAACGAGCTC
Setaria         TGGGACGACATGGAGAAGATTTGGCATCACACCTTCTACAACGAGCTC
Populus         TGGGATGATATGGAAAAGATATGGCATCATACCTTCTACAATGAGCTT

cons            ***** ** ***** ***** ******** ** ******** *****

                                Primer region candidate

Elaeis          CGTGTTGCCCCTGAGGAGCACCCTGTGCTGCTCACTGAGGCCCCTCTC
Hordeum         CGTGTCGCCCCAGAGGAGCACCCCGTCCTTCTCACTGAGGCGCCGCTC
Oryza           CGTGTGGCCCCGGAGGAGCACCCCGTCCTCCTCACCGAGGCTCCTCTC
S.vulgare.      CGTGTGGCTCCCGAGGAGCACCCCGTCCTCCTCACTGAGGCGCCCCTG
Zea             CGTGTGGCTCCCGAGGAACACCCCGTCCTCCTCACTGAGGCGCCCCTG
Setaria         CGTGTCGCGCCCGAGGAGCACCCCGTGCTGCTGACCGAGGCCCCCCTG
Populus         CGTGTTGCCCCAGAAGAGCATCCAGTGCTCCTAACTGAGGCTCCTCTG

cons            ***** ** ** ** ** ** ** ** ** ** ** ***** ** **


Elaeis          AACCCCAAGGCAAACAGAGAGAAGATGACCCAAATCATGTTTGAAACA
Hordeum         AACCCCAAGGCCAATCGTGAGAAGATGACCCAGATCATGTTCGAGACC
Oryza           AACCCCAAGGCCAATCGTGAGAAGATGACCCAGATCATGTTTGAGACC
S.vulgare.      AACCCCAAGGCTAACCGTGAGAAGATGACCCAGATCATGTTCGAGACC
Zea             AACCCAAAGGCTAACAGGGAGAAGATGACCCAGATCATGTTCGAGACC
Setaria         AACCCCAAGGCTAACAGGGAGAAGATGACCCAGATCATGTTTGAGACA
Populus         AACCCCAAGGCTAATCGTGAGAAGATGACTCAGATCATGTTTGAGACC

cons            ***** ***** **  * ********** ** ******** ** **
```

```
Elaeis          TTCAATGTACCTGCCATGTATGTTGCAATCCAAGCAGTTCTATCACTA
Hordeum         TTCAACACTCCTGCTATGTATGTCGCCATCCAGGCCGTCCTCTCGCTG
Oryza           TTCAACACCCCTGCTATGTACGTCGCCATCCAGGCCGTCCTCTCTCTG
S.vulgare.      TTCAACACCCCCGCCATGTACGTCGCCATCCAGGCCGTCCTCTCTCTG
Zea             TTCAACACCCCCGCTATGTACGTCGCCATCCAGGCCGTCCTGTCTCTG
Setaria         TTCAATGTGCCGGCCATGTATGTCGCCATTCAGGCTGTGCTTTCCCTC
Populus         TTCAACACTCCTGCTATGTATGTTGCCATTCAGGCTGTCCTGTCCTTG

cons            *****     ** ** ***** ** ** ** ** ** ** ** **  *

Elaeis          TATGCTAGTGGTCGTACAACTGGTATTGTTCTTGACTCGGGAGATGGT
Hordeum         TATGCCAGTGGTCGTACCACAGGTATTGTGCTGGACTCGGGAGATGGT
Oryza           TATGCCAGTGGTCGTACCACAGGTATTGTGTTGGACTCTGGTGATGGT
S.vulgare.      TATGCCAGCGGTCGTACCACAGGTATCGTGCTCGACTCGGGAGATGGT
Zea             TATGCCAGTGGTCGTACCACAGGTATCGTGCTCGACTCGGGAGATGGT
Setaria         TACGCCAGTGGACGCCACAACAGGTATCGTGTTGGACTCTGGTGATGGT
Populus         TATGCCAGTGGTCGTACAACTGGTATTGTGTTGGACTCTGGTGATGGT

cons            ** ** ** ** ** ** ** ***** **  * ***** ** ******

Elaeis          GTTACCCACACTGTCCCAATTTATGAGGGATTTGCACTTCCTCATGCC
Hordeum         GTCAGCCACACTGTCCCCATCTACGAAGGATACGCTCTTCCCCACGCT
Oryza           GTCAGCCACACTGTCCCCATCTATGAAGGATATGCTCTCCCCCATGCT
S.vulgare.      GTCAGCCACACTGTCCCCATCTACGAAGGGTACGCCCTCCCCCACGCC
Zea             GTGAGCCACACCGTCCCCATCTACGAGGGATACGCCCTCCCCCACGCC
Setaria         GTGAGCCATACCGTGCCAATCTATGAAGGTTATGCCCTTCCGCACGCC
Populus         GTCAGCCATACAGTCCCCATATATGAGGGGTATGCCCTTCCACATGCC

cons            ** * *** ** ** ** ** ** ** ** *  ** ** ** ** **

Elaeis          ATCCTTCGATTGGATCTTGCTGGCCGTGATCTCACTGATGCTTTGATG
Hordeum         ATCCTCCGTCTTGACCTGGCTGGGCGTGATCTCACCGATTACCTCATG
Oryza           ATCCTTCGTCTCGACCTTGCTGGGCGTGATCTCACTGATTACCTCATG
S.vulgare.      ATCCTGCGTCTCGACCTCGCTGGCCGCGACCTTACCGACTACCTCATG
Zea             ATCCTTCGTCTCGACCTGGCTGGCCGCGACCTCACCGACTACCTCATG
Setaria         ATTCTCCGTCTTGACCTTGCTGGACGTGATCTCACTGACAGTCTGATG
Populus         ATCCTTCGTCTTGACCTGGCTGGCCGTGACCTCACTGATTCCTTGATG

cons            ** ** **  * ** ** ***** ** ** ** ** **      * ***


Elaeis          AAGATACTTACTGAGAGAGGCTATTCTTTCACCACCACTGCAGAGCGG
Hordeum         AAGATCCTCACTGAGCGTGGTTACTCATTCACCACCACTGCTGAGCGG
Oryza           AAGATCCTGACGGAGCGTGGTTACTCATTCACCACAACGGCCGAGCGG
S.vulgare.      AAGATCCTGACTGAGCGCGGCTACTCCTTCACCACCACTGCTGAGCGG
Zea             AAGATCCTGACTGAACGCGGCTACTCCTTCACCACCACTGCTGAGCGG
Setaria         AAGATTCTTACTGAGAGGGGTTACTCCTTCACCACCACTGCCGAGCGG
Populus         AAGATCCTCACTGAGCGTGGTTATTCTTTCACAACCACAGCTGAACGG

cons            ***** ** **  **  * ** ** ** ***** ** ** ** ** ***
```

```
Elaeis          GAAATTGTTAGGGATATAAAGGAGAAACTTGCTTATGTTGCCCTCGAT
Hordeum         GAAATTGTGAGGGATGTGAAGGAGAAGCTGTCCTACATTGCACTGGAC
Oryza           GAAATTGTGAGGGACATGAAGGAGAAGCTTTCCTACATCGCCCTGGAC
S.vulgare.      GAAATTGTCAGGGACATGAAGGAGAAGCTCGCCTACATTGCCCTGGAC
Zea             GAAATTGTCAGGGACATGAAGGAGAAGCTCGCCTACATTGCCCTGGAC
Setaria         GAAATTGTAAGAGACATCAAGGAGAAGCTCGCCTATGTGGCACTTGAC
Populus         GAAATTGTGAGGGACATGAAGGAAAAGCTAGCTTACATTGCTCTTGAC

cons            ******** ** **  * ***** ** **  * **  * ** ** **

Elaeis          TATGAGCAGGAATTGGAGTCTGCCAAGAGCAGCTCCTCTGTAGAAAGG
Hordeum         TACGACCAGGAAATGGAGACTTCCAAGACCAGCTCTTCTGTGGAGAAG
Oryza           TATGACCAGGAAATGGAGACTGCCAAGACCAGCTCCTCCGTGGAGAAG
S.vulgare.      TACGACCAGGAGATGGAGACTGCCAAGACCAGCTCTTCCGTGGAGAAG
Zea             TACGACCAGGAGATGGAGACTGCCAAGACCAGCTCTTCTGTTGAGAAG
Setaria         TATGAGCAGGAGCTTGAGACTGCTAAGACCAGCTCCTCTGTGGAGAAG
Populus         TACGAGCAGGAGCTAGAGACCTCCAAGACCAGCTCAGCAGTTGAAAAG

cons            ** ** *****  * *** *  * **** ******  * ** ** * *

Elaeis          AGTTATGAGCTGCCTGATGGGCAGGTCATCACCATTGGTGCAGAGAGA
Hordeum         AGCTACGAGCTTCCTGATGGGCAGGTTATCACCATTGGTTCCGAGCGT
Oryza           AGCTACGAGCTTCCTGATGGACAGGTTATCACCATTGGTGCTGAGCGT
S.vulgare.      AGCTACGAGCTTCCTGATGGACAGGTCATCACCATTGCGGCGGACCGA
Zea             AGCTACGAGCTGCCTGACGGACAGGTCATCACCATTGGTGCTGAGCGC
Setaria         AGCTATGAGCTGCCTGATGGGCAGGTGATCACCATCGGGGCAGAGAGG
Populus         AGCTATGAATTGCCTGATGGGCAGGTCATCACCATTGGTGCTGAACGT

cons            ** ** **  * ***** ** ***** ******** *   * **  *


Elaeis          TTCAGGTGTCCAGAGGTTCTTTTCCAGCCATCCCTGATTGGAATGGAA
Hordeum         TTCCGTTGCCCTGAGGTCCTCTTCCAGCCATCCTTCATCGGGATGGAA
Oryza           TTCCGCTGCCCTGAGGTCCTCTTCCAGCCTTCCTTCATAGGAATGGAA
S.vulgare.      TTCCGCTGCCCTGAGGTCCTCTTCCAGCCATCCTTCATTGGGATGGAA
Zea             TTCCGCTGCCCTGAGGTCCTCTTCCAGCCATCCTTCATTGGGATGGAA
Setaria         TTCAGATGCCCTGAGGTCCTTTTCCAGCCTTCATTCATTGGTATGGAG
Populus         TTCCGTTGCCCAGAGGTCCTCTTCCAACCATCAATGATCGGAATGGAA

cons            *** * ** ** ***** ** ***** ** **  * ** ** *****


Elaeis          GCTGCTGGAATCCATGAAACTACCTACAATTCCATCATGAAGTGTGAT
Hordeum         GCTGCAGGTATCCATGAGACCACCTACAACTCCATCATGAAGTGTGAC
Oryza           GCTGCGGGTATCCATGAGACTACATACAACTCCATCATGAAGTGCGAC
S.vulgare.      GCTGCTGGCATTCACGAGACTACCTACAACTCCATCATGAAGTGCGAC
Zea             GCTGCTGGTATCCACGAGACCACCTACAACTCCATCATGAAGTGCGAC
Setaria         TCGCCTGGAATCCATGAGACCACCTACAACTCTATCATGAAGTGTGAT
Populus         GCAGCAGGCATCCACGAGACCACATACAACTCCATCATGAAGTGTGAT

cons            *  * ** ** ** ** ** ** ***** ** ********** **
```

```
Elaeis          GTGGATATCAGGAAGGACTTGTATGGTAACGTTGTGCTCAGTGGAGGA
Hordeum         GTGGATATTAGGAAGGATCTGTACGGCAACATTGTTCTTAGTGGTGGT
Oryza           GTGGATATTAGGAAGGATCTATATGGCAACATCGTTCTCAGTGGTGGT
S.vulgare.      GTGGATATTAGGAAGGATCTATATGGCAACATCGTCCTCTCTGGTGGT
Zea             GTGGATATTAGGAAGGATCTGTATGGCAACATCGTCCTCTCCGGTGGT
Setaria         GTGGATATTAGGAAGGACCTCTATGGTAACATTGTGCTCAGCGGTGGC
Populus         GTCGATATCAGGAAAGACTTGTATGGTAACATTGTCCTCAGTGGTGGT

cons            ** ***** ***** **  * ** ** *** * ** **    ** **

Elaeis          TCAACCATGTTCCCTGGTATTGCTGATCGTATGA-GCAAGGAAATCTC
Hordeum         ACCACTATGTTCACTGGAATCGCTGATAGGATGA-GCAAGGAGATCAC
Oryza           ACCACTATGTTCCCTGGCATTGCTGACAGGATGA-GCAAGGAGATCAC
S.vulgare.      ACCACTATGTTCCCTGGGATTGCTGACAGGATGAGGCAAGGAAATCAC
Zea             ACCACTATGTTCCCTGGCATTGCTGACAGGATGA-GCAAGGAAATCAC
Setaria         TCAACCATGTTCCCTGGTATTGCTGACCGCATGA-GCAAGGAGATCAC
Populus         TCAACTATGTTCCCAGGAATTGCTGACAGAATGA-GCAAGGAAATCTC

cons            * ** ****** * ** ** *****  * **** ******* *** *

Elaeis          GGCCCTTGCTCCAAGCAGCATGAAGATTAAAGTTGTCGCTCCACCCGA
Hordeum         TGCCTTGGCTCCTAGCAGCATGAAGATTAAGGTTGTTGCTCCTCCTGA
Oryza           TGCCTTGGCTCCTAGCAGCATGAAGATCAAGGTGGTCGCCCCTCCTGA
S.vulgare.      TGCC-TTGCTCCTAGCAGCATGAAGATCAAGGTGGTTGCTCCTCCAGA
Zea             CGCCCTGGCTCCTAGCAGCATGAAGATCAAGGTGGTTGCTCCTCCAGA
Setaria         TGCCCTTGCACCAAGCAGTATGAAGATTAAGGTGGTGGCACCACCTGA
Populus         TGCACTAGCCCCAAGCAGCATGAAAATCAAGGTGGTTGCACCACCAGA

cons            **  * ** ** ***** ***** ** ** ** ** ** ** ** **

Elaeis          ACGGAAGTATTCTGTTTGGATTGGTGGTTCTATCCTTGCATCCCTCAG
Hordeum         AAGGAAGTACAGTGTCTGGATCGGAGGATCCATCTTGGCATCTCTCAG
Oryza           AAGGAAGTACAGTGTCTGGATTGGAGGATCCATCTTGGCATCTCTCAG
S.vulgare.      AAGGAAGTACAGTGTCTGGATTGGAGGATCCATCTTGGCATCTCTCAG
Zea             AAGGAAGTACAGTGTCTGGATTGGAGGATCCATCCTGGCATCGCTCAG
Setaria         GAGGAAATACAGTGTCTGGATTGGAGGGTCCATCCTTGCCTCCCTTAG
Populus         AAGGAAATACAGTGTCTGGATTGGTGGTTCAATCTTGGCATCCCTTAG

cons              **** **    *** ***** ** ** ** *** * ** ** ** **

Elaeis          CACCTTCCAGCAGATGTGGATTTCAAAGGAAGAGTATGATGAATGTGG
Hordeum         CACCTTCCAGCAGATGTGGATTGCAAAGGCTGAGTACGACGAGTCTGG
Oryza           CACATTCCAGCAGATGTGGATTGCCAAGGCTGAGTACGACGAGTCTGG
S.vulgare.      CACATTCCAGCAGATGTGGATTGCCAAGGCTGAGTACGACGAGTCTGG
Zea             CACCTTCCAGCAGATGTGGATTGCCAAGGCTGAGTACGACGAGTCTGG
Setaria         CACCTTCCAACAGATGTGGATCTCGAAGGGTGAGTATGATGAGTCAGG
Populus         CACCTTCCAGCAGATGTGGATTGCAAAGGCAGAGTATGACGAGTCAGG

cons            *** ***** **********  * ****  ***** ** ** *  **
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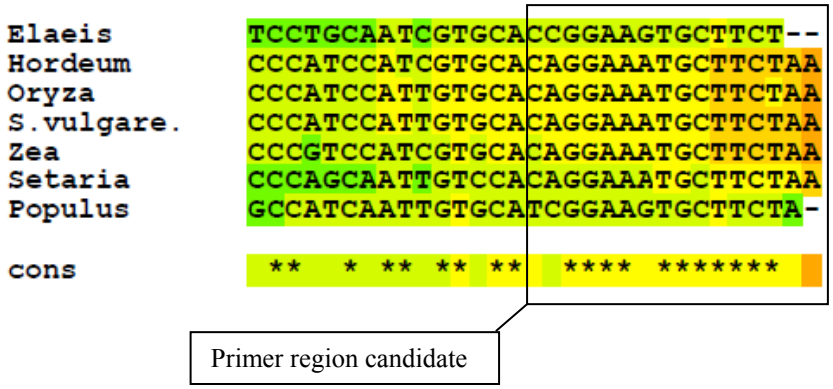
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Hordeum CCCATCCATCGTGCACAGGAAATGCTTCTAA
Oryza CCCATCCATTGTGCACAGGAAATGCTTCTAA
S.vulgare. CCCATCCATTGTGCACAGGAAATGCTTCTAA
Zea CCCGTCCATCGTGCACAGGAAATGCTTCTAA
Setaria CCCAGCAATTGTCCACAGGAAATGCTTCTAA
Populus GCCATCAATTGTGCATCGGAAGTGCTTCTA-
cons ** * ** ** ** **** *******
Primer region candidate

Pal1 Nucleotide Alignment

```
CLUSTAL 2.1 multiple sequence alignment


Zea                 GACCCGCTGAACTGGGGCGCGGCGGCGGCGGAGCTGGCCGGGAGCCACCTGGACGAGGTG 60
Saccharum           GACCCGCTGAACTGGGGCGCGGCGGCAGCGGAGCTGGCGGGGAGCCACCTGGACGAGGTG 60
Oryza               GACCCGCTCAACTGGGGCGCGGCGGCGGCCGAGATGGCCGGCAGCCACCTCGACGAGGTG 60
Phyllostachys       GACCCGCTTAACTGGGGGAAGGCGGCGGAGGAACTGATGGGGAGCCACTTGGATGAGGTG 60
Bambusa             GACCCGCTTAACTGGGGGAAGGCGGCGGAGGAGCTGATGGGGAGCCATTTGGACGAGGTG 60
Triticum            GACCCACTCAACTGGGGGAAGGCGGCGGAGGAGCTCTCGGGTAGCCATTTGGAGGCGGTG 60
                    ***** ** ********   ****** *  **  *    ** *****  * ** * ****

Zea                 AAGCGCATGGTGGCGCAGGCCCGGCAGCCCGTGGTCAAGATCGAGGGCTCCACCCTCCGC 120
Saccharum           AAGCGCATGGTGGCGCAGGCCCGGCAGCCCGTGGTGAAGATCGAGGGCTCCACGCTCCGC 120
Oryza               AAGCGCATGGTGGCGCAGTTCCGCGAGCCGCTGGTCAAGATCCAGGGCGCCACCCTCCGC 120
Phyllostachys       AAGAGGATGGTCACGGAATACCGCCAACCCGTGGTGAAGATCGAGGGCGCCAGCCTGAGG 120
Bambusa             AAGCGGATGGTGGCGGAGTACCGCCAGCCGGTGGTGAAGATCGAGGGCGCCAGCCTGAGG 120
Triticum            AAGCGGATGGTGGAGGAGTACCGCAAGCCGGTCGTGACGATGGAGGGCGCCA---CGACC 117
                    *** * *****   * *   ***  * **  * ** * ***  ***** ***

Zea                 GTCGGCCAGGTGGCCGCCGTCGCCTCCGCCAAGGACGCGTCCGGCGTCGCCGTCGAGCTC 180
Saccharum           GTCGGCCAGGTGGCCGCCGTCGCCGCCGCCAAGGACGCGTCGGGCGTCGCCGTCGAGCTC 180
Oryza               GTCGGCCAGGTGGCCGCCGTCGCCCAGGCCAAGGACGCCGCGCGCGTCGCCGTCGAGCTC 180
Phyllostachys       ATCGCGCAGGTCGCGGCAGTGGCCGCGGCCGGCGAGGC---------CAAGGTGGAGCTC 171
Bambusa             ATCGCTCAGGTCGCGGCAGTGGCCGTGGCCGGCGACGC---------CAAGGTGGAGCTC 171
Triticum            ATCGCGATGGTCGCCGCGGTGGCTGCCGGCAGCGACAC---------CAGAGTGGAGCTG 168
                     ***    *** ** ** ** **    * *   **  *         *   ** *****

Zea                 GACGAGGAGGCCCGCCCCCGCGTCAAGGCCAGCAGCGAGTGGATCCTCGACTGCATCGCC 240
Saccharum           GACGAGGAGGCCCGCCCCCGCGTCAAGGCCAGCAGCGAGTGGATCCTCGACTGCATCGCC 240
Oryza               GACGAGGAGGCCCGCCCCCGCGTCAAGGCCAGCAGCGAGTGGATCCTCACCTGCATCGCC 240
Phyllostachys       GACGAGTCGGCTCGCGAACGCGTCAAGGCCAGTAGCGACTGGGTCATGAACAGCATGATG 231
Bambusa             GACGAGTCGGCTCGTGAACGCGTCAAGGCCAGCAGCGACTGGGTCATGAACAGCATGATG 231
Triticum            GACGAGTCCGCCCGCGGCCGCGTCAAGGAGAGCAGCGACTGGGTCATGAACAGCATGATG 228
                    ******   ** **    **********  ** ***** *** ** *   * ****

Zea                 CACGGCGGCGACATCTACGGCGTCACCACCGGCTTCGGCGGCACCTCCCACCGCCGCACC 300
Saccharum           CACGGCGGCGACATCTACGGCGTCACCACCGGCTTCGGCGGCACCTCCCACCGCCGCACC 300
Oryza               CACGGCGGCGACATCTACGGCGTCACCAC-GGCTTCGGCGGCACCTCCCACCGCCGCACC 299
Phyllostachys       AACGGCACCGACAGCTACGGTGTCACCACCGGCTTCGGTGCCACATCCCACCGGAGGACC 291
Bambusa             AACGGCACCGACAGCTACGGTGTCACCACCGGCTTCGGTGCCACATCGCACAGGAGGACC 291
Triticum            AACGGCACCGACAGTTACGGTGTCACCACCGGCTTCGGCGCCACCTCTCACCGGAGGACC 288
                    *****  *****  ***** ******** ******** * *** ** *** *  * ***

Primer region candidate

Zea                 AAGGACGGGCCCGCGCTCCAGGTCGAGCTGCTCAGGCATCTCAACGCCGGAATCTTCGGC 360
Saccharum           AAGGACGGGCCCGCTCTCCAGGTCGAGCTGCTCAGGCATCTCAACGCCGGAATCTTCGGC 360
Oryza               AAGGACGGCCCCGCCCTCCAAGTCGAGCTCCTCAGGTATCTCAACGCCGGAATCTTCGGC 359
Phyllostachys       AAGGAGGGTGGTGCTCTCCAAAGGGAGCTCATCAGATTCCTCAATGCCGGCGCGTTTGGC 351
Bambusa             AAGGAGGGTGGTGCTCTCCAGAGGGAGCTCATCAGATTCCTCAACGCCGGCGCCTTCGGC 351
Triticum            AAGGAGGGCGGCGCTCTCCAGAGAGAGCTCATCCGATTCCTTAACGCGGGAGCCTTCGGC 348
                    ***** **    ** *****    *****  ** *    ** ** ** **    ** ***

Zea                 ACCGGCAGCGACGGGCACACGCTGCCGTCGGAGGTCACCCGCGCGGCGATGCTGGTGCGC 420
Saccharum           ACCGGCAGCGATGGCCACACGCTGCCGTCGGAGGTCGTCCGCGCGGCGATGCTGGTGCGC 420
Oryza               ACTGGCTCCGATGGCCACACGCTGCCGTCGGAGACGGTGCGGGCGGCCATGCTCGTGCGC 419
Phyllostachys       ACTGGCACCGACAGCCATGTTCTGCCTGCTGCGGCAACCCGTGCGGCCATGCTCGTCCGC 411
Bambusa             ACTGGCTGCGACGGCCACGTTCTGCCGGCCGAGGCAACTCGAGCGGCCATGCTCGTCCGC 411
Triticum            ACCGGCACCGACGGCCACGTTCTGCCTGCCGCAGCGACAAGGGCGGCGATGCTCGTCCGA 408
                    ** ***  ***  * **    *****  * *         * ***** ***** ** **
```

```
Zea               ATCAACACCCTCCTCCAGGGCTACTCCGGCATCCGCTTCGAGATCCTCGAGGCCATCACG 480
Saccharum         ATCAACACCCTCCTCCAGGGCTACTCGGGCATCCGCTTCGAGATCCTGGAGGCCATCACC 480
Oryza             ATCAACACCCTCCTCCAGGGCTACTCCGGCATCCGGTTCGAGATCCTCGAGGCCATCACC 479
Phyllostachys     ATCAACACTCTCCTTCAAGGATACTCCGGAATCCGGTTCGAGATCCTCGAGGCGATTGCC 471
Bambusa           ATCAACACCCTCCTCCAGGGCTATTCCGGAATCCGCTTCGAGATCCTCGAGGCCATCACC 471
Triticum          GTCAATACCTTGCTCCAGGGATACTCAGGGATCCGCTTCGAGATCCTCGAGACGATCGCC 468
                   **** **  * ** ** ** ** ** ** ***** ********** *** * **  *

Zea               AAGCTGCTCAACACCGGTGTCAGCCCCTGCCTGCCGCTCCGGGGCACCATCACCGCGTCG 540
Saccharum         AAGCTGCTCAACACCGGGGTCAGCCCGTGCCTGCCGCTCCGGGGCACCATCACCGCGTCG 540
Oryza             AAGCTGCTCAACACCGGCGTCACGCCGTGCCTGCCGCTCCGTGGCACCATCACCGCGTCC 539
Phyllostachys     AAGCTGCTCAATGCCAATGTCACGCCGTGCCTACCGCTCCGGGGCACGATCACCGCGTCC 531
Bambusa           AAGCTGCTTAATGCCAACGTCACGCCGTGCCTGCCGCTCAGGGGCACGGTCACCGCGTCC 531
Triticum          ACGCTTCTCAACGCCAACGTGACACCTTGCCTGCCGCTCCGGGGCACGATCACCGCGTCG 528
                  * *** ** **  **   ** *  ** ***** ****** * *****  **********

Zea               GGCGACCTGGTCCCGCTCTCCTACATCGCCGGCCTCATCACGGGCCGCCCCAACGCGCAG 600
Saccharum         GGCGACCTCGTCCCGCTCTCCTACATCGCCGGCCTCATCACGGGCCGCCCCAACGCGCAG 600
Oryza             GGTGACCTGGTTCCCCTGTCCTACATTGCCGGCCTCATCACCGGCCGCCCCAACGCGCAG 599
Phyllostachys     GGTGACCTGGTCCCGCTGTCCTACATTGCTGGCCTCGTCACCGGCCGCGAGAACTCTGTT 591
Bambusa           GGCGACCTGGTCCCGCTCTCATACATTGCCGGCCTTGTCACCGGCCGCGAGAACTCTGTT 591
Triticum          GGTGACCTCGTCCCGCTTTCCTACATCGCCGGCCTGGTCACCGGCCGCCCAAACTCCATG 588
                  ** ***** ** ** ** ** ***** ** *****  **** ******   *** *

Zea               GCCGTCACCGTCGACGGAAGGAAGGTGGACGCCGCCGAGGCGTTCAAGATCGCCGGCATC 660
Saccharum         GCCACCACCGTCGACGGGAGGAAGGTGGACGCCGCCGAGGCGTTCAAGATCGCCGGCATC 660
Oryza             GCCATCTCGCCCGACGGCAGGAAGGTGGACGCCGCCGAGGCGTTCAAGCTCGCCGGCATC 659
Phyllostachys     GCTGTCACCCCTGATGGCAGGAAGGTGAACGCCGCCGAGGCGTTCAAGATTGCCGGCATC 651
Bambusa           GCTGTCGCCCCCGACGGCAGGAAGGTGAACGCCGCTGAGGCGTTTAAAATTGCCGGCATC 651
Triticum          GCGACGGCTCCGGATGGTTCGAAGGTTAATGCTGCGGAGGCATTTAAGATCGCCGGCATC 648
                  **     *    ** **   ******  * ** ** ***** ** **  * *********

Zea               GAGGGCGGCTTCTTCAAGCTCAACCCCAAGGAGGGCCTCGCCATCGTCAACGGCACGTCC 720
Saccharum         GAGGGCGGCTTCTTCAAGCTCAACCCCAAGGAAGGTCTCGCCATCGTCAACGGCACCTCC 720
Oryza             GAGGGTGGCTTCTTCACGCTGAACCCCAAGGAAGGTCTCGCCATCGTCAATGGCACGTCC 719
Phyllostachys     CAGGGCGGCTTCTTCGAGTTGCAGCCCAAGGAAGGCCTTGCCATGGTGAACGGTACAGCT 711
Bambusa           CAGGGCGGCTTCTTCGAGTTGCAGCCTAAGGAAGGTCTGGCCATGGTCAACGGCACTGCC 711
Triticum          CAGCACGGCTTCTTCGAGCTACAGCCCAAGGAAGGCCTTGCCATGGTGAATGGCACGGCA 708
                   **   *********  * *  * ** ***** ** ** ***** ** ** ** **  *

Zea               GTGGGCTCCGCGCTCGCGGCCACCGTGATGTACGACGCCAACGTCCTGGCCGTCCTGTCG 780
Saccharum         GTGGGCTCCGCGCTCGCGGCCACCGTGATGTACGACGCCAACGTCCTCACCGTCCTGTCC 780
Oryza             GTGGGGTCGGCGCTCGCGGCCACCGTGATGTTCGACGCCAACATCCTCGCCGTCCTGTCC 779
Phyllostachys     GTGCGCTCCGGTCTCGCATCGACCGTGCTCTTTGAGGCGAACATTCTTGCCATCCTTGCC 771
Bambusa           GTGGGCTCTGGACTTGCCTCCACGGTGCTCTTTGAAGCGAACATACTTGCAATCCTCGCC 771
Triticum          GTGGGCTCAGGCCTTGCCTCCATGGTGCTTTTCGAGGCAAACGTCCTTAGCCTCCTTGCT 768
                  *** * ** *  ** **  * *  *** * *  ** ** *** * **     ****  *

Zea               GAGGTCCTGTCCGCCGTCTTCTGCGAGGTCATGAACGGCAAGCCCGAGTACACGGACCAC 840
Saccharum         GAGGTCCTGTCCGCCGTCTTCTGCGAGGTGATGAACGGCAAGCCCGAGTACACCGACCAC 840
Oryza             GAGGTGCTCTCGGCGGTGTTCTGCGAGGTGATGAACGGCAAGCCGGAGTACACCGACCAC 839
Phyllostachys     GAGGTCCTGTCCGCCGTGTTCTGCGAGGTTATGAACGGCAAGCCGGAGTACACCGACCAC 831
Bambusa           GAGGTCCTGTCCGCCGTGTTCTGCGAAGTCATGAACGGCAAGCCGGAGTACACCGACCAC 831
Triticum          GAGGTCTTGTCGGGCGTCTTCTGTGAGGTCATGAACGGCAAGCCGGAGTTCACCGACCAC 828
                  *****  * ** *  ** ***** ** ** ** ************** **** *** ******
```

Primer region candidate

```
Zea                CTGACCCACAAGCTGAAGCACCACCCGGGGTCCATCGAGGCCGCGGCCATCATGGAGCAC 900
Saccharum          CTCACCCACAAGCTCAAGCACCACCCGGGGTCCATCGAGGCCGCCGCCATCATGGAGCAC 900
Oryza              CTGACCCACAAGCTGAAGCACCACCCTGGGTCGATCGACGCCGCCGCCATCATGGAGCAC 899
Phyllostachys      CTGACCCACAAGCTGAAGCACCACCCGGGACAAATCGAGGCTGCTGCTATAATGGAGCAC 891
Bambusa            CTTACGCACAAGCTGAAGCATCATCCTGGACAGATAGAGGCTGCTGCTATCATGGAGCAC 891
Triticum           TTGACCCATAAGTTGAAGCACCACCCCGGGCAAATTGAGGCCGCCGCCATCATGGAGCAC 888
                    * ** ** *** * ***** ** ** **    ** ** ** ** ** ** *********

Zea                ATCCTGGATGGCAGCTCCTTCATGAAGCAGGCCAAGAAGGTGAACGAGCTGGACCCGCTG 960
Saccharum          ATCCTGGACGGCAGCGCCTTCATGAAGCACGCCAAGAAGGTGAACGAGCTGGACCCGCTG 960
Oryza              ATCCTCGCCGGGAGCTCGTTCATGAGCCACGCCAAGAAGGTGAACGAGATGGACCCGCTG 959
Phyllostachys      ATCTTGGAGGGAAGCTCCTACATGAAGCTTGCTAAGAAGCTTGGCGAGCTCGACCCACTG 951
Bambusa            ATCTTGGAGGGAAGCTCATACATGAAGCTTGCTAAGAAGCTCGGTGACCTCGACCCGTTG 951
Triticum           ATCCTTGAAGGCAGCTCCTACATGATGCTCGCAAAGAAGCTCGGTGAGCTTGACCCACTG 948
                   *** * *  ** *** * * *****  *  ** ****** *    **  * *****  **

Zea                CTGAAGCCCAAGCAGGACAGGTACGCGCTCCGCACGTCGCCGCAGTGGCTGGGCCCCCAG 1020
Saccharum          CTCAAGCCCAAGCAGGACAGGTACGCGCTCCGCACGTCGCCGCAGTGGCTGGGCCCCCAG 1020
Oryza              CTGAAGCCGAAGCAGGACAGGTACGCGCTCCGCACGTCGCCGCAGTGGCTCGGCCCGCAG 1019
Phyllostachys      ATGAAGCCAAAGCAAGACCGGTACGCGCTCAGAACATCCCCGCAGTGGCTCGGCCCGCAA 1011
Bambusa            ATGAAGCCAAAACAGGACCGCTACGCGCTCCGCACGTCGCCGCAATGGCTCGGCCCCCAA 1011
Triticum           ATGAAGCCAAAGCAAGATAGGTATGCACTCCGCACATCGCCGCAGTGGCTTGGCCCTCAG 1008
                    * ***** ** ** **  * ** ** *** * ** ** ***** ***** ***** **

Zea                ATCGAGGTCATCCGCGCCGCCACCAAGTCCATCGAGCGCGAGGTCAACTCCGTGAACGAC 1080
Saccharum          ATCGAGGTCATCCGCGCCGCCACCAAGTCCATCGAGCGCGAGGTCAACTCCGTCAACGAC 1080
Oryza              ATCCAGGTCATCCGCGCCGCCACCAAGTCCATCGAGCGCGAGGTCAACTCCGTGAACGAC 1079
Phyllostachys      ATTGAGGTTATCCGTGCAGCCACCAAGTCCATTGAGCGCGAGATCAACTCCGTCAATGAC 1071
Bambusa            ATTGAGGTTATCCGTGCCGCCACCAAGTCCATTGAGCGCGAGATCAACTCTGTCAACGAC 1071
Triticum           ATTGAGGTCATCCGTGCTGCCACCAAGTCAATCGAGCGTGAGATCAATTCCGTCAACGAC 1068
                   **  **** ***** ** ********** ** ***** *** **** ** ** ** ***

Zea                AACCCGGTCATCGACGTCCACCGCGGCAAGGCGCTGCACGGCGGCAACTTCCAGGGCACC 1140
Saccharum          AACCCGGTCATCGACGTCCACCGTGGCAAGGCGCTGCACGGCGGCAACTTCCAGGGCACG 1140
Oryza              AACCCGGTGATCGACGTCCACCGCGGCAAGGCGCTCCACGGCGGCAACTTCCAGGGCACC 1139
Phyllostachys      AACCCACTCATCGATGTCTCCCGCGGCAAGGCGATTCACGGTGGCAACTTCCAGGGTACG 1131
Bambusa            AACCCGCTCATTGACGTCTCCCGCAATAAGGCGCTTCACGGTGGCAACTTCCAGGGCACG 1131
Triticum           AACCCACTCATCGATGTCTCCCGCGGCAAAGCTATCCATGGTGGCAACTTCCAAGGCACG 1128
                   *****  * ** ** ***  ***    ** **  * ** ** ********** ** **

Zea                CCCATCGGCGTGTCCATGGACAACGCCCGCCTCGCCATCGCCAACATCGGCAAGCTCATG 1200
Saccharum          CCCATCGGCGTGTCCATGGACAACGCTCGCCTCGCCATCGCCAACATCGGCAAGCTCATG 1200
Oryza              CCCATCGGTGTGTCCATGGACAACGCCCGTCTCGCCATCGCCAACATCGGCAAGCTCATG 1199
Phyllostachys      CCCATCGGCGTTTCCATGGACAACACCCGCCTCGCCATTGCCGCCATCGGCAAGCTGATG 1191
Bambusa            CCCATCGGTGTGTCCATGGACAACACCCGCCTCGCCATTGCTGCCATCGGCAAGCTCATG 1191
Triticum           CCCATCGGTGTGTCCATGGACAACACCAGGCTTGCCATTGCAGCGATCGGCAAGCTCATG 1188
                   ******** ** ************ *  * ** ***** **    ************ ***

Zea                TTCGCGCAGTTCTCCGAGCTCGTCAACGAGTTCTACAACAACGGGCTCACCTCCAACCTG 1260
Saccharum          TTCGCGCAGTTCTCGGAGCTGGTCAACGAGTTCTACAACAACGGGCTCACCTCCAACCTG 1260
Oryza              TTCGCGCAGTTCTCCGAGCTCGTGAACGAGTTCTACAACAACGGGCTGACCTCCAACCTG 1259
Phyllostachys      TTCGCGCAGTTCTCAGAACTCGTGAACGACTTCTACAACAACGGCCTGCCTTCCAACCTG 1251
Bambusa            TTTGCGCAGTTTTCGGAGCTCGTGAACGACTTCTACAACAATGGCCTTCCCTCCAACCTG 1251
Triticum           TTTGCCCAGTTCTCGGAGCTGGTGAACGACTTCTACAACAACGGTCTGCCTTCCAACCTC 1248
                   ** ** ***** ** ** ** ** ***** ********** ** **  * *******
```

Primer region candidate

```
Zea              GCCGGCAGCCGCAACCCCAGCCTGGACTACGGCTTCAAGGGCACCGAGATCGCCATGGCC 1320
Saccharum        GCCGGCAGCCGCAACCCCAGCCTGGACTACGGCTTCAAGGGCACGGAGATCGCCATGGCC 1320
Oryza            GCCGGCAGCCGCAACCCGAGCTTGGACTACGGGTTCAAGGGCACCGAGATCGCCATGGCC 1319
Phyllostachys    TCCGGCGGGCGCAACCCGAGCTTGGACTACGGCTTCAAGGGCGCCGAGATCGCCATGGCC 1311
Bambusa          TCCGGCGGGCGCAACCCGAGCTTGGACTACGGTTTCAAGGGCGCCGAGATCGCCATGGCC 1311
Triticum         TCCGGCGGGCGCAACCCAAGCTTGGACTATGGCTTCAAGGGTGCCGAGATTGCCATGGCC 1308
                  ***** * ******** *** ******* ** ********  * ***** *********

Zea              TCCTACTGCTCCGAGCTCCAGTACCTGGGCAACCCCATCACCAACCACGTGCAGAGCGCG 1380
Saccharum        TCCTACTGCTCTGAGCTGCAGTACCTGGGCAACCCCATCACCAACCACGTNCAGAGCGCG 1380
Oryza            TCCTACAGCTCTGAGCTCCAGTACCTCGCCAACCCCATCACCAACCATGTCCAGAGCGCG 1379
Phyllostachys    TCGTACCGCTCTGAGCTGCAGTTCTTGGGCAACCCGGTGACTAACCACGTCCAGAGCGCC 1371
Bambusa          TCGTACTGCTCTGAGCTGCAGTTCTTGGGCAACCCGGTGACGAACCACGTCCAGAGCGCG 1371
Triticum         TCGTACTGCTCCGAGCTCCAATTCTTAGGCAACCCTGTGACCAACCATGTTCAGAGCGCG 1368
                 ** *** **** ***** ** * * * * ******  * ** ***** ** ********

Zea              GACGAGCACAACCAGGACGTGAACTCCCTGGGCCTCGTCTCGGCCAGGAAGACCGCCGAG 1440
Saccharum        GAGCAGCACAACCAGGACGTCAACTCCCTCGGCCTCGTCTCCGCCAGGAAGACCGCCGAG 1440
Oryza            GAGCAGCACAACCAGGACGTGAACTCGCTGGGTCTCGTCTCGGCCAGGAAGACCCTGGAG 1439
Phyllostachys    GAGCAGCACAACCAGGACGTCAACTCTCTTGGTCTCATCTCTTCCAGGAAGACCGCCGAG 1431
Bambusa          GAGCAACACAACCAGGACGTCAATTCCCTTGGTCTCATCTCCTCCAGGAAGACCGCCGAG 1431
Triticum         GAGCAACACAACCAAGATGTCAACTCTCTTGGTCTCATCTCCTCAAGGAAGACTGCAGAG 1428
                 **  * ******** ** ** ** ** ** ** *** ****  * ********    ***

Zea              GCGATCGACATCCTGAAGCTCATGTCGTCCACCTACATCGTGGCGCTGTGCCAGGCCGTG 1500
Saccharum        GCCATCGACATCCTGAAGCTCATGTCGTCCACCTACATCGTGGCGCTGTGCCAGGCCATC 1500
Oryza            GCGGTGGACATCCTCAAGCTCATGACCTCCACCTACATCGTCGCCCTGTGCCAGGCCGTC 1499
Phyllostachys    GCCATCGACATCTTGAAGCTCATGTCCTCGACCTTCTTGGTCGCCCTGTGCCAGGCCATC 1491
Bambusa          GCCATCGACATCCTGAAGATCATGTCCTCGACGTTCTTGGTCGCCTTGTGCCAGGCCATC 1491
Triticum         GCCATTGACATATTGAAGCTCATGTCCTCAACATTCTTGGTCGCGTTGTGCCAGGCTATC 1488
                 **  * *****  * *** ***** * ** ** * * * ** **  **********  *

Zea              GACCTGCGCCACCTCGAGGAGAACATCAAGGCGTCGGTGAAGAACACCGTGACCCAGGTG 1560
Saccharum        GACCTGCGCCACCTCGAGGAGAACATCAAGACGTCGGTGAAGAACACGGTGACCCAGGTG 1560
Oryza            GACCTTCGCCACCTCGAGGAGAACATCAAGAGCTCCGTCAAGAACTGCGTCACCCAGGTG 1559
Phyllostachys    GACCTTCGCCACATCGAGGAGAATGTCAAGAGCGCCGTCAAGAGCTGCGTCATGACAGTG 1551
Bambusa          GACCTGCGCCACATCGAGGAGAACGTCAAAAGCGCCGTCAAGAGCTGCGTTATGACAGTG 1551
Triticum         GACCTCCGCCACCTTGAGGAGAATGTCAAGAATGCTGTCAAGAGCTGCGTGAAGACAGTG 1548
                 ***** ****** * ********  ****     * ** **** *   ** *     ***

Zea              GCCAAGAAGGTGCTGACCATGAACCCCTCGGGCGAGCTCTCCAGCGCCCGCTTCAGCGAG 1620
Saccharum        GCGAAGAAGGTGCTGACCATGAACCCGTCGGGCGACCTCTCCAGCGCGCGCTTCAGCGAG 1620
Oryza            GCCAAGAAGGTGCTCACCATGAACCCCACCGGCGACCTCTCCAGCGCGCGCTTCAGCGAG 1619
Phyllostachys    GCCAAGAAGACTCTGAGCACCAACTCCACCGGTGATCTTCACGTCGGTCGCTTCTGCGAG 1611
Bambusa          GCCAAGAAGACTCCGAGCACCAACTCCACCGGTGATCTTCACGTCGCTCGCTTCTGCGAG 1611
Triticum         GCTAGGAAGACACTGAGCACTGATAACAATGGCCATCTCCACAACGCACGCTTCTGCGAG 1608
                 ** * ****   *  * **   *       **  * **   *  **  ****** *****

Zea              AAGGAGCTGATCAGCGCCATCGACCGCGAGGCCGTGTTCACGTACGCGGAGGACGCGGCC 1680
Saccharum        AAGGAGCTCATCACCGCCATCGACCGCGAGGGCGTGTTCACCTACGCGGAGGACCCGGCC 1680
Oryza            AAGAACCTCCTCACCGCCATCGACCGCGAGGCCGTGTTCAGCTATGCCGACGACCCGTGC 1679
Phyllostachys    AAGGACCTGCTAAAGGAGATTGACTGTGAGGCGGTGTTCGCGTACGCCGACGACCCGTGC 1671
Bambusa          AAGGACCTGCTCAAGGAGATCGACCGTGAGGCGGTGTTCGCGTACGCCGACGACCCATGC 1671
Triticum         AAGGACCTTCTGCTCACAATCGACCGTGAGGCCGTGTTCGCGTACGCAGATGACCCCTGC 1668
                 *** * **  *       ** *** * ****  ******   ** ** ** *** *   *
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```
Zea              AGCGCCAGCCTGCCGCTGATGCAGAAGCTGCGCGCCGTGCTGGTGGACCACGCCCTCAGC 1740
Saccharum        AGCGGCAGCCTGCCGCTGATGCAGAAGCTGCGCTCCGTGCTGGTGGACCACGCCCTCAGC 1740
Oryza            AGCGCCAACTACCCGCTCATGCAGAAGCTCCGCGCCGTGCTCGTCGAGCACGCCCTCACC 1739
Phyllostachys    TGCCCCAACTACCCACTGATGAAGAAGATGCGCAATGTGCTCGTGGAGCGCGCCCTTGCT 1731
Bambusa          AGCCCCAACTACCCACTGATGAAGAAGCTGCGCAGTGTGCTCGTGGAGAGCGCCCTCGCC 1731
Triticum         AGCGCCAACTACCCCCTCATGCAGAAGATGCGTGCAGTTCTCGTGGAGCACGCCTTGGCC 1728
                  **  ** *   ** ** *** ***** * **    ** ** ** **   **** *

Zea              AGCGGCGAGCGCGG-AGCGG-----GAGCCCTCCGTGTTCTCCAAGATCACCAGGTTCGA 1794
Saccharum        AGCGGCGA-CGCGGGAACGG-----GAGCCCTCCGTGTTCTCCAAGATCACCAATTTCGA 1794
Oryza            AGCGGCGACCGCCG-AGCCC-----GAGGCCTCCGTGTTCTCCAAGATCACCAAGTTCGA 1793
Phyllostachys    AACGGCATG-GCCGAGTTCAATGCAGAGACCTCCGTGTTTGCCAAGGTTGCCCAGTTCGA 1790
Bambusa          AACGGCATG-GCCGAGTTCAATGCAGAGACTTCTATATTTGCCAGGGTCGCCCTGTTCGA 1790
Triticum         AATGGTGAG-GCCGAGGCGCACGTCGAGACGTCGGTGTTTGCCAAGCTTGCCATGTTCGA 1787
                 *  **     ** *           *** * **  * **  *** * *  **   *****

Zea              GGAGGAGCTCCGCGCGGTGCTGCCCCAGGAGGTGGAGGCCGCCCGCGTGGC-GTCG---- 1849
Saccharum        GGAGGAGCTCCGCGCGGGGCTGGCCCGGGAGGTGGAAGGCGCCC-CGCTTC-GCCGTGGG 1852
Oryza            GGAGGAGCTCCGCTCTGCGCTGCCCCGGGAGATCGAGGCCGCCCGCGTCGCCGTCG---- 1849
Phyllostachys    GGAGGAGTTGCGCGCGGCGCTGCCCAGGGCGGTTGAGGCCGCACGGGCAGCTGTGGAG-- 1848
Bambusa          GGAGGAACTACGCGCGGCGCTGCCCAGGGCAGTCGAGGCTGCACGGGCGTCAGTCGAG-- 1848
Triticum         GCAGGAGCTCCGTGCAGTGTTGCCAAAGGAGGTCGAGGCCGCCCGAAGCGCCGTGGAG-- 1845
                 * ****  * **  * * * ** *   **   * ** *  ** *      * *  *

Zea              CCGAGGGCACCGCCCCCGTGGCGAA-CCGGATCGCGGACAGCCGGTCGTTCCCGCTGTAC 1908
Saccharum        CCGAGGGCACCGCCCCCG-GGCGAAACCGGAACTGGGACAGCCGGTCGTTCCCGCTGTAC 1911
Oryza            -CCAACGCACCGCCCCCGTCGCCAA-CCGGATCGTCGAGAGCCGGTCGTTCCCGCTCTAC 1907
Phyllostachys    --AACGGCACGGCAGC-ATTACCCAACAGAATCACTGAGTGCCGCTCGTACCCGCTCTAC 1905
Bambusa          --AACGGCACGGCAGC-AGCACCCAACAGAATCACCGAGTGCCGGTCGTATCCCCTGTAC 1905
Triticum         --AATGGCACCGCAGC-ACAGCAAAACCGTATCGCCGAATGTCGGTCGTACCCGCTCTAC 1902
                    *  **** **  *     *  * * * * *   **  * ** ****  ** ** ***

Zea              CGCTTCGTGCGCGAGGAGCTCGGCTGCGTGTTCCTGACCGGCGAGAGGCTCAAGTCCCCC 1968
Saccharum        CGCTTCGTCCGCGAGGAGCTCGGCTGCGTGTTCCTGACCGGCGAGAAGCTCAAGTCCCCC 1971
Oryza            CGCTTCGTCCGCGAGGAGCTCGGCTGCGTATTCCTCACCGGCGAGAAGCTCAAGTCCCCC 1967
Phyllostachys    CGGTTTGTGCGTGAGGAGCTCGGAGCCGCATACCTCACCGGCGAGAAGACACGGTCGCCC 1965
Bambusa          CGGTTTGTACGCGAGGAGCTCGGGACGGAGTACCTCACCGGCGAGAAGACACGGTCGCCG 1965
Triticum         CGGTTCGTGCGCAAGGAGCTTGGAACGGAGTACTTGACCGGGGAGAAGACGCGGTCTCCT 1962
                 ** ** ** **  ******* **    *  * * * ***** **** *     *** **

Zea              GGCGAGGAGTGCAACAAGGTGTTCGTCGGCATCAGCCAGGGCAAGCTCGTGGACCCCATG 2028
Saccharum        GGCGAGGAGTGCACCAAGGTGTTCAACGGCATCAGCCAGGGCAAGCTCGTCGACCCCATG 2031
Oryza            GGCGAGGAGTGCAACAAGGTGTTCCTCGGCATCAGCCAGGGCAAGCTCATCGACCCCATG 2027
Phyllostachys    GGCGAGGAGCTGAACAAGGTGCTCGTGGCCATCAACCAGGGCAAGCACATCGACCCGCTG 2025
Bambusa          GGCGAGGAGCTGAACAAGGTGCTCCTCGCCATCAACCAGGGCAAGCACATTGACCCGCTG 2025
Triticum         GGCGAAGAGGTGGACAAGGTGTTCGTTGCCATGAACCAAGGCAAGCACATCGACGCGCTG 2022
                 ***** ***     ******* **   * *** * *** ******* * * *** *  **

Zea              CTCGAGTGCCTCAAGGAGTGGGACGGCAAGCCGCTGCCCATCAAC 2073
Saccharum        CTCGAGTGCCTCAAGGAGTGGGACGGCAAGCCGCTGCCCATCAAC 2076
Oryza            CTCGACTGCCTCAAGGAGTGGAACGGCGAGCCCCTTCCCATCAAC 2072
Phyllostachys    CTCGAGTGCCTCAAGGAGTGGAACGGCG-GCCACTGCCCATCTGC 2069
Bambusa          CTTGAGTGCCTCAAGGAGTGGAACGGCGAGCCACTGCCCATCTGC 2070
Triticum         CTGGAGTGCCTCAAGGAGTGGAACGGCGAGCCCCTGCCTCTCTGC 2067
                 ** ** ********************  *** ** **  **  *
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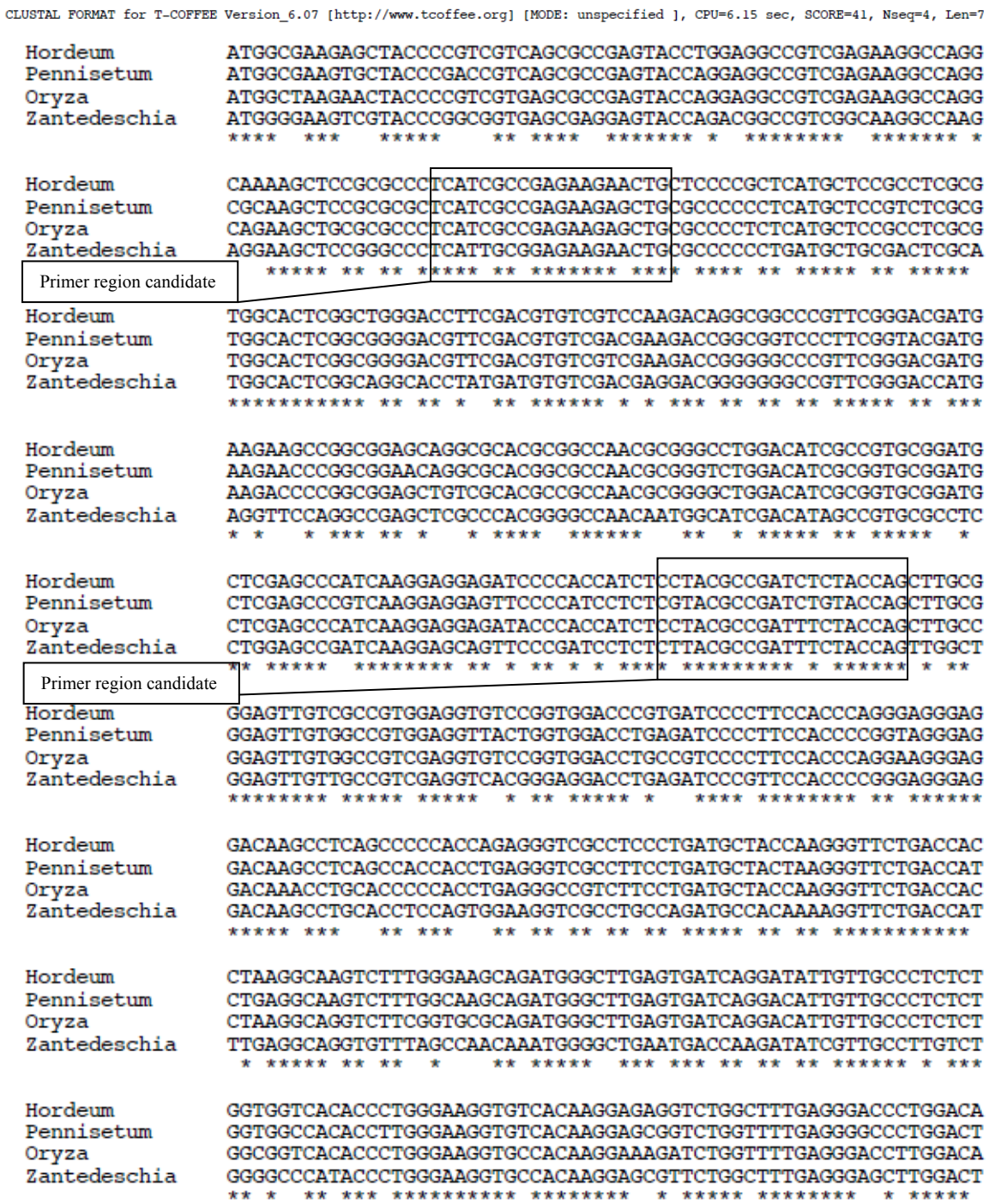
Primer region candidate

Apx1 Nucleotide Alignment



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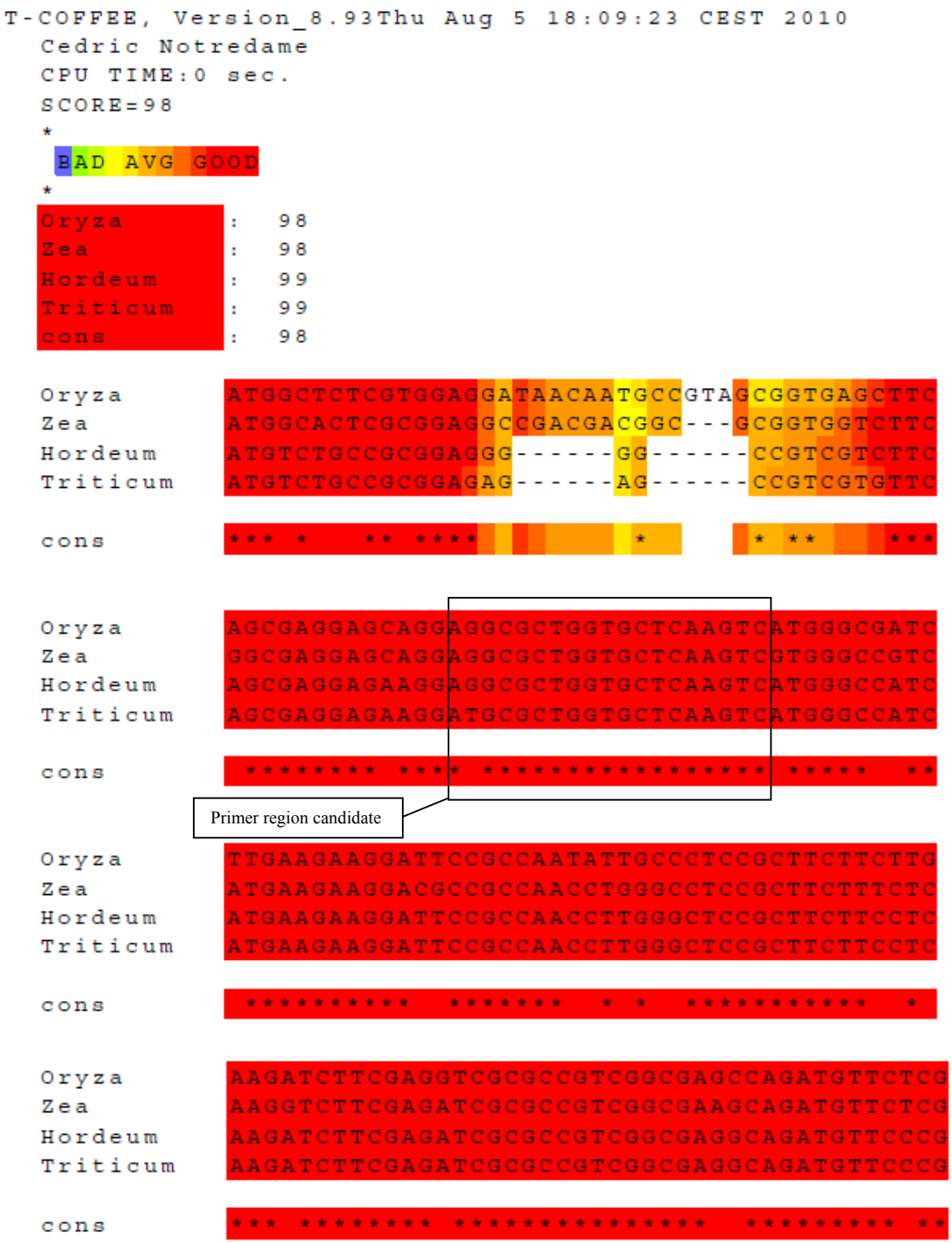
Hordeum          AGGAACCCTTTGAAGTTTGACAACTCTTACTTCACGGAGCTTTTGAGTGGTGACAAAGAG
Pennisetum       AGAAACCCTTTGGTCTTTGACAACTCTTACTTCAAGGAACTTCTGACCGGTGACAAGGAG
Oryza            AGAAACCCTCTGCAGTTTGACAACTCTTACTTCACGGAGCTTCTGAGTGGTGACAAGGAG
Zantedeschia     ACTAATCCTCTCATCTTTGATAACTCCTACTTCAAGGAGCTTCTGTCCGGCGAGAAGGAA
                 *  ** *** *    ***** ***** ******* *** *** **   ** ** ** **

Hordeum          GGACTTCTTCAGCTTCCAAGTGACAAAACTCTGCTGACTGACCCTGTCTTCCGCCCTCTT
Pennisetum       GGCCTCCTTCAGCTCCCAAGTGACAAAACTCTGCTGAGTGACCCTGTCTTCCGCCCTCTT
Oryza            GGCCTTCTTCAGCTTCCTAGTGACAAAGCCCTGCTGAGTGACCCTGCCTTCCGCCCACTC
Zantedeschia     GACCTTCTCCAGCTGCCTTCTGACAAAGCTCTCCTCAGTGATCCTGTGTTCCGCCCGCTT
                 *  ** ** ***** **   ******* * ** ** * *** ****  ******** **

Hordeum          GTGGAGAAATATGCTGCGGATGAGAAGGCTTTCTTCGAGGACTACAAGGAGGCACACCTC
Pennisetum       GTGGAGAAATATGCTGCGGATGAGAAGGCTTTCTTTGATGACTACAAGGAGGCCCACCTC
Oryza            GTCGAGAAATATGCTGCAGATGAGAAGGCTTTCTTTGAAGACTACAAGGAGGCCCACCTC
Zantedeschia     GTGGAGAAATACGCAGCTGACGAAGATGCTTTCTTTGCCGACTATACTGAAGCTCACTTG
                 ** ******** ** ** ** **  * ******** *  ***** *  ** ** *** *

Hordeum          AGGCTCTCCGAACTGGGGTACGCTGA-AGCCTAA
Pennisetum       AGGCTCTCTGAACTGGGGTTCGCTGA-TGCATAA
Oryza            AAGCTCTCCGAACTGGGGTTCGCTGA-TGCTTAA
Zantedeschia     AAGCTCTCCGAGCTTGGGTTTGCCGAGTGTT-GA
                 * ****** ** ** ****  ** **  *    *
```

Hb1 Nucleotide Alignment



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Oryza       TTCCTGCGCAACTCCGACGTGCCGCTCGAGAAGAACCCCAAG
Zea         TTCCTGCGCGACTCCGACGTGCCGCTGGAGAAGAACCCCAAG
Hordeum     TTCCTGCGCGACTCCGACGTGCCGCTGGAGACCAACCCCAAG
Triticum    TTCCTGCGCGACTCCGACGTGCCGCTGGAGACCAACCCCAAG

cons        ********* ***************** ****  *********

Oryza       CTCAAGACCCACGCCATGTCCGTCTTCGTCATGACATGCGAG
Zea         CTCAAGACGCACGCCATGTCCGTCTTCGTCATGACCTGCGAG
Hordeum     CTCAAGACCCACGCCGTGTCCGTCTTCGTCATGACCTGCGAG
Triticum    CTCAAGACCCACGCCGTGTCCGTCTTCGTCATGACGTGTGAG

cons        ******** ****** ******************** ** ***


Oryza       GCCGCCGCGCAGCTGCGGAAAGCCGGGAAGGTCACCGTGAGA
Zea         GCGGCGGCGCAGCTTCGCAAGGCCGGGAAGGTCACCGTGAGG
Hordeum     GCGGCTGCGCAGTTGCGGAAAGCCGGCAAGATCACCGTCAGG
Triticum    GCGGCAGCCCAGCTGCGGAAAGCCGGGAAGATCACCGTGAGG

cons        ** ** ** *** * ** ** ***** *** ******* **


Oryza       GACACCACCCTCAAGAGGCTCGGCGCCACGCACCTCAAGTAC
Zea         GAGACCACGCTCAAGAGGCTGGGCGCCACGCACTTGAGGTAC
Hordeum     GAGACCACCCTGAAGAGGCTGGGCGGCACGCACTTGAAATAC
Triticum    GAGACCACCCTGAAGAGGCTGGGCGGGACGCACTTGAAATAC

cons        ** ***** ** ******** ****  ****** * *  ***


Oryza       GGCGTCGGAGACGCCCACTTCGAGGTGGTGAAGTTCGCGCTG
Zea         GGCGTCGCAGATGGACACTTCGAGGTGACGGGGTTCGCGCTG
Hordeum     GGCGTGGCAGATGGCCACTTCGAGGTGACGCGGTTCGCTCTG
Triticum    GGCGTGGCGGATGGCCACTTTGAGGTGACGCGGTTCGCTCTG

cons        ***** *  ** *  ***** ******  *  ****** ***


Oryza       CTTGACACGATCAAGGAGGAGGTTCCGGCGGACATGTGGAGC
Zea         CTTGAGACGATCAAGGAGGCGCTCCCCGCTGACATGTGGAGC
Hordeum     CTCGAGACGATCAAGGAGGCGCTTCCGGCTGACATGTGGGGG
Triticum    CTCGAGACGATCAAGGAGGCGCTTCCGGCGGACATGTGGGGG

cons        ** ** ************ * * ** ** ********* *
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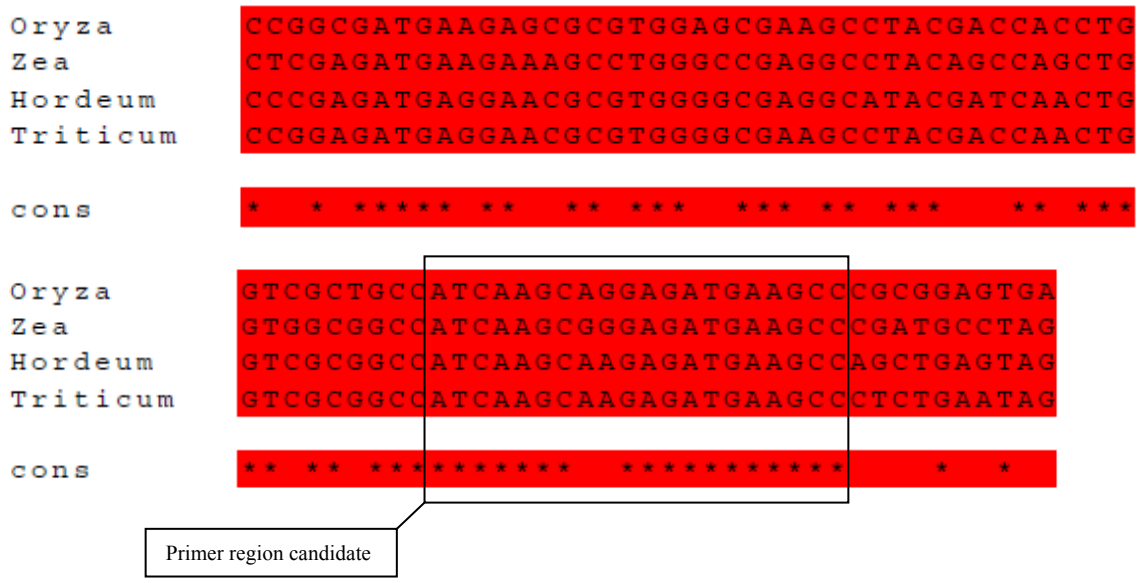
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Zea CTCGAGATGAAGAAAGCCTGGGCCGAGGCCTACAGCCAGCTG
Hordeum CCCGAGATGAGGAACGCGTGGGGCGAGGCATACGATCAACTG
Triticum CCGGAGATGAGGAACGCGTGGGGCGAAGCCTACGACCAACTG
cons
Oryza GTCGCTGCCATCAAGCAGGAGATGAAGCCCGCGGAGTGA
Zea GTGGCGGCCATCAAGCGGGAGATGAAGCCCGATGCCTAG
Hordeum GTCGCGGCCATCAAGCAAGAGATGAAGCCAGCTGAGTAG
Triticum GTCGCGGCCATCAAGCAAGAGATGAAGCCCTCTGAATAG
cons
Primer region candidate

APPENDIX B

TABLE I. *Species used for amino acid alignments for* Act1 *and* Pal1

Species	Common Name	Monocot/Dicot	NCBI Reference Number
Actin Alignment			
Brassica oleracea	cabbage	D	AAD02328.1
Brassica rapa	field mustard	D	AAZ67555.1
Coleochaete scutata	freshwater algae	-	AAC16054.1
Gossypium hirsutum	cotton	D	AAP73453.1
Isatis tinctoria	Dyer's woad	D	AAW63030.1
Linum usitatissimum	flax	D	AAW34192.1
Musa acuminata	dessert banana	M	ABS11262
Oryza sativa	rice	M	Swiss-Prot: Q10DV7
Physcomitrella patans	moss	-	AAQ88111.1
Pisum sativum	pea	D	AAB18644.1
Solanum tuberosum	potato	D	CAA39279.1
Vallisneria natans	freshwater aquatic plant-eelgrass	M	AAF40477
Zea mays	corn	M	Swiss-Prot: P02582
Pal1 Alignment			
Allium cepa	onion	M	AAS48415
Bambusa oldhamii	bamboo	M	AAR24505
Bromheadia finlaysoniana	orchid	D	Swiss-Prot: Q42609
Hordeum vulgare	barley	M	Swiss-Prot: O04876
Isatis tinctoria	Dyer's woad	D	ABF50788.1
Lithospermum erythrorhizon	stone seed	D	Swiss-Prot: O49836
Lotus japonicus	lotus	D	BAF36970.1
Oryza sativa	rice	M	Swiss-Prot: P14717
Petroselinum crispum	parsley	D	PDB: 1W27
Phyllostachys edulis	tortoise shell bamboo	M	ABP96954
Populus tremuloides	quaking aspen	D	AAN52280.1
Saccharum officinarum	sugarcane	M	ABM63378
Solanum tuberosum	potato	D	Swiss-Prot: P31425
Trifolium pratense	red clover	D	AAZ29733.1
Trifolium subterraneum	subterranean clover	D	2006271A
Triticum aestivum	wheat	M	Swiss-Prot: Q43210
Zea mays	corn	M	Swiss-Prot: Q8VXG7

TABLE II. *Species used for amino acid alignments for* Apx1 *and* Hb1

Species	Common Name	Monocot/Dicot	NCBI Reference Number
Apx1 Alignment			
Arabidopsis thaliana	thale cress	D	Swiss-Prot: Q05431
Elaeis guineensis	African oil palm	M	ACF06591.1
Hordeum vulgare	barley	M	CAA06996.1
Oryza sativa	rice	M	Swiss-Prot: Q10N21
Pennisetum glaucum	pearl millet	M	ABP65326.1
Zantedeschia aethiopica	common arum lily	M	AAC08576.1
Hb1 Alignment			
Hordeum vulgare	barley	M	AAB70097.1
Oryza sativa	rice	M	Swiss-Prot: O04986
Triticum aestivum	wheat	M	AAN85432.1
Zea mays	corn	M	Swiss-Prot: Q9FY42

TABLE III. *Species used for nucleotide alignments for* Act1 *and* Pal1

Species	Common Name	Monocot/Dicot	NCBI Reference Number
Actin Alignment			
Elaeis guineensis	African oil palm	M	AY550991.1
Hordeum vulgare	barley	M	AK251023.1
Oryza sativa	rice	M	NM_001057621.1
Populus trichocarpa	black cottonwood	D	EF44345.1
Setaria italica	foxtail bristlegrass	M	AF288226.1
Sorghum bicolor	sorghum	M	X79378.1
Zea mays	corn	M	AY107106.1
Pal1 Alignment			
Bambusa oldhamii	bamboo	M	AAR24505
Oryza sativa	rice	M	CAA34226
Phyllostachys edulis	tortoise shell bamboo	M	ABP96954
Saccharum officinarum	sugarcane	M	ABM63378
Triticum aestivum	wheat	M	AY005474.1
Zea mays	corn	M	L77912.1

TABLE IV. *Species used for nucleotide alignments for* Act1 *and* Pal1

Species	Common Name	Monocot/Dicot	NCBI Reference Number
Apx1 Alignment			
Hordeum vulgare	barley	M	AJ006358.1
Oryza sativa	rice	M	D45423.1
Pennisetum glaucum	pearl millet	M	EF495352.1
Zantedeschia aethiopica	common arum lily	M	AF053474.1
Hb1 Alignment			
Hordeum vulgare	barley	M	U94968.1
Oryza sativa	rice	M	U76030.1
Triticum aestivum	wheat	M	AAN85432.1
Zea mays	corn	M	AY005818.1

Table V. *Summary of conditions used for optimization for each primer set*

Primer set	Amount of Genomic DNA	Annealing Temp. Range (°C)	Primer Concentration (μM)	Number of PCR Cycles
Pal1-1	10-100 ng	47-62	0.25-0.5	30-35
Pal1-2	10-100 ng	47-62	0.25-0.5	30-35
Pal1-3	10-100 ng	55-65	0.25-0.5	30-35
Apx1	10-100 ng	45-58	0.25-0.5	30-35
Hb1	10-100 ng	44-55	0.25-0.5	30-35
Act1	10-100 ng	47-57	0.25-0.5	30-35

TABLE VI. *Plant actin sequences used to construct phylogenetic tree*

Species	Common Name	Monocot/Dicot	NCBI Reference Number
Actinidia deliciosa	kiwifruit	D	ABR45727.1
Arabidopsis thaliana	thale cress	D	NP_001031504.1
Betula platyphylla	Asian whitebirch	D	ACB88021.1
Caragana korshinskii	peashrub	D	ACK87035.1
Coleochaete scutata	freshwater algae	-	AF061019.1
Gossypium hirsutum	cotton	D	AAP73453.1
Guzmania wittmackii x Guzmania lingulata	bromeliad	M	ADN88106.1
Gynura bicolor	Okinawa spinach	D	BAJ17659.1
Helianthus annuus	sunflower	D	ACL27886.1
Helianthus annuus	sunflower	D	ACL27885.1
Hordeum vulgare	barley	M	AAN59956.1
Jatropha curcas	Barbados nut	D	ADH82414.1
Litchi chinensis	lychee	D	ADV17460.1
Magnolia denudata	lilytree	D	AAF87302.1
Malva pusilla	low mallow	D	AAD41039.1
Morus alba	white mulberry	D	ADU52547.1
Musa acuminata	dessert banana	M	ABS11262
Nicotiana tabacum	tobacco	D	BAD27408.1
Oryza sativa	rice	M	BAC76319.1
Persea americana	avocado	D	ADA70361.1
Phalaenopsis hybrid	orchid	M	AAN08622.1
Physcomitrella patans	moss	-	XP_001783901.1
Picea abies	Norway spruce	-	ACP19072.1
Populus trichocarpa	black cottonwood	D	XP_002322664.1
Populus trichocarpa	black cottonwood	D	XP_002308365.1
Stevia rebaudiana	stevia	D	AAN40685.1
Thellungiella halophila	salt cress	D	BAJ34498.1
Tulipa gesneriana	Didier's tulip	M	BAH98157.1
Vallisneria natans	freshwater aquatic plant-eelgrass	M	AAF40477
Vitis vinifera	wine grape	D	XP_002282516.1
Zea mays	corn	M	J01238.1

TABLE VII. *Plant GAPDH sequences used to construct phylogenetic tree*

Species	Common Name	Monocot/Dicot	NCBI Reference Number
Acontinum noveboracense	northern monkshood	D	ACN25135.1
Arabidopsis thaliana	thale cress	D	NP_187062.1
Atriplex nummularia	Australian saltbrush	D	AAA03442.1
Begonia bowerae	eyelash begonia	D	ACU27390.1
Beta vulgaris	beet	D	ABN50381.1
Brassica napus	rape seed	D	ACS68203.1
Capsicum annuum	chili pepper	D	CAC80375.1
Coleochaete scutata	freshwater algae	-	DQ873409.1
Cucumis melo	cantaloupe	D	ADN33957
Dalea purpurea	purple prairie-clover	D	ADK20403.1
Daucus carota	carrot	D	AAR84410.2
Dieffenbachia seguine	dumb cane	M	ACT34014.1
Dionaea muscipula	Venus flytrap	D	GQ249157.2
Ginko biloba	maidenhair tree	-	AAA33352.1
Glycine max	soybean	D	ABA07956.1
Gossypium hirsutum	cotton	D	ACJ11752.1
Guzmania wittmackii x Guzmania lingulata	bromeliad	M	HM185058.1
Hordeum vulgare	barley	M	Swiss-Prot: P26517.1
Lilium longiflorum	trumpet lily	M	DQ318775.1
Lupinus albus	white lupine	D	CAI83772.1
Magnolia lilliflora	purple magnolia	D	CAA42905.1
Mesembryanthemum crystallinum	common iceplant	D	AAA33031.1
Musa acuminata	dessert banana	M	AAV70659.1
Nicotiana tabacum	tobacco	D	CAB39974.1
Oryza sativa	rice	M	ADM86845.1
Physcomitrella patans	moss	-	EDQ52052.1
Phyllostachys edulis	bamboo	M	ADB98096.1
Pilea cadierei	aluminum plant	D	GQ332381.1
Pisum sativum	pea	D	AAA33667.1
Ricinus communis	castor bean	D	EEF51837.1
Solanum chacoense	chacopotato	D	ACV69976.1
Solanum lycopersicon	tomato	D	AAB54003.1
Taxus baccata	English yew (conifer)	-	Swiss-Prot: Q41595.1
Thymus vulgaris	Thyme	D	HM153755.1
Tradescantia padilla	spiderwort	M	ADL67550.1
Triticum aestivum	wheat	M	ABQ81648.1
Zea mays	corn	M	ACG36109.1

APPENDIX C

le VIII. *Actin genomic sequence descriptions for* T. testudinum, H. engelmannii, and Ruppia maritima

Bank ession nber	Source	Clone ID	Organism	Species Authors	Common Name	Collection Locality	Coordinates	Collection Da (Month-Yr.)
2035	blade/genomic DNA	1-Tt	*Thalassia testudinum*	J. Blanks & D. Solander *ex* Köenig	Turtle Grass	Corpus Christi, TX	27°47'28.07"N, 97°7'22.16"W	Jul-08
2036	blade/genomic DNA	2-Tt	*Thalassia testudinum*	J. Blanks & D. Solander *ex* Köenig	Turtle Grass	Corpus Christi, TX	27°47'28.07"N, 97°7'22.16"W	Jul-08
2037	blade/genomic DNA	6-Tt	*Thalassia testudinum*	J. Blanks & D. Solander *ex* Köenig	Turtle Grass	Corpus Christi, TX	27°47'28.07"N, 97°7'22.16"W	Jul-08
2038	blade/genomic DNA	7-Tt	*Thalassia testudinum*	J. Blanks & D. Solander *ex* Köenig	Turtle Grass	Corpus Christi, TX	27°47'28.07"N, 97°7'22.16"W	Jul-08
2039	Mixed rhizome and blade/genomic DNA	1,4,5,7,8,9,14 (-He)	*Halophila engelmannii*	P. Asherson	Star Grass or Peanut Grass	Corpus Christi, TX	27°39'08.41"N, 97°16'40.78"W	Jul-08
9825	rhizome/genomic DNA	1-Rm, 3-Rm	*Ruppia maritima*	C. Linneaus	Wigeongrass	Corpus Christi, TX	27°39'08.41"N, 97°16'40.78"W	Jul-08
9826	rhizome/genomic DNA	2-Rm, 4-Rm, 5-Rm	*Ruppia maritima*	C. Linneaus	Wigeongrass	Corpus Christi, TX	27°39'08.41"N, 97°16'40.78"W	Jul-08

le IX. *Actin genomic sequence descriptions for* H. beaudettei *and* C. filiformis

Bank ession nber	Source	Clone ID	Organism	Species Authors	Common Name	Collection Locality	Coordinates	Collection Da (Month-Yr.)
6857	rhizome/genomic DNA	2-Hw	*Halodule beaudettei*	(C. den Hartog) C. den Hartog	Shoal Grass	Corpus Christi, TX	27°39'08.41"N, 97°16'40.78"W	Jul-08
6858	rhizome/genomic DNA	4-Hw	*Halodule beaudettei*	(C. den Hartog) C. den Hartog	Shoal Grass	Corpus Christi, TX	27°39'08.41"N, 97°16'40.78"W	Jul-08
6859	rhizome/genomic DNA	5-Hw	*Halodule beaudettei*	(C. den Hartog) C. den Hartog	Shoal Grass	Corpus Christi, TX	27°39'08.41"N, 97°16'40.78"W	Jul-08
6860	rhizome/genomic DNA	7-Hw	*Halodule beaudettei*	(C. den Hartog) C. den Hartog	Shoal Grass	Corpus Christi, TX	27°39'08.41"N, 97°16'40.78"W	Jul-08
2678	Mixed rhizome and blade/genomic DNA	1-Sf	*Cymodocea filiformis*	(F. Kützing) D. Correll	Manatee Grass	Corpus Christi, TX	27°39'08.41"N, 97°16'40.78"W	Jul-08
2679	Mixed rhizome and blade/genomic DNA	2-Sf	*Cymodocea filiformis*	(F. Kützing) D. Correll	Manatee Grass	Corpus Christi, TX	27°39'08.41"N, 97°16'40.78"W	Jul-08
2680	Mixed rhizome and blade/genomic DNA	3-Sf	*Cymodocea filiformis*	(F. Kützing) D. Correll	Manatee Grass	Corpus Christi, TX	27°39'08.41"N, 97°16'40.78"W	Jul-08
2681	Mixed rhizome and blade/genomic DNA	5-Sf	*Cymodocea filiformis*	(F. Kützing) D. Correll	Manatee Grass	Corpus Christi, TX	27°39'08.41"N, 97°16'40.78"W	Jul-08

le X. *Actin cDNA sequence descriptions for* H. beaudettei

Bank ession mber	Source	Clone ID	Organism	Species Authors	Common Name	Collection Locality	Coordinates	Collection Da (Month-Yr.)
75761	rhizome/RNA extraction→cDNA	1	*Halodule beaudettei*	(C. den Hartog) C. den Hartog	Shoal Grass	Corpus Christi, TX	27°44'15.76"N, 97°08'18.22W	Aug-10
75762	rhizome/RNA extraction→cDNA	2	*Halodule beaudettei*	(C. den Hartog) C. den Hartog	Shoal Grass	Corpus Christi, TX	27°44'15.76"N, 97°08'18.22W	Aug-10
75763	rhizome/RNA extraction→cDNA	3	*Halodule beaudettei*	(C. den Hartog) C. den Hartog	Shoal Grass	Corpus Christi, TX	27°44'15.76"N, 97°08'18.22W	Aug-10
75764	rhizome/RNA extraction→cDNA	4	*Halodule beaudettei*	(C. den Hartog) C. den Hartog	Shoal Grass	Corpus Christi, TX	27°44'15.76"N, 97°08'18.22W	Aug-10
75765	rhizome/RNA extraction→cDNA	5	*Halodule beaudettei*	(C. den Hartog) C. den Hartog	Shoal Grass	Corpus Christi, TX	27°44'15.76"N, 97°08'18.22W	Aug-10
75766	rhizome/RNA extraction→cDNA	6	*Halodule beaudettei*	(C. den Hartog) C. den Hartog	Shoal Grass	Corpus Christi, TX	27°44'15.76"N, 97°08'18.22W	Aug-10
75767	rhizome/RNA extraction→cDNA	8	*Halodule beaudettei*	(C. den Hartog) C. den Hartog	Shoal Grass	Corpus Christi, TX	27°44'15.76"N, 97°08'18.22W	Aug-10
75768	rhizome/RNA extraction→cDNA	10	*Halodule beaudettei*	(C. den Hartog) C. den Hartog	Shoal Grass	Corpus Christi, TX	27°44'15.76"N, 97°08'18.22W	Aug-10

le XI. *GAPDH genomic and cDNA sequence descriptions for* H. beaudettei

Bank ession mber	Source	Clone ID	Organism	Species Authors	Common Name	Collection Locality	Coordinates	Collection Da (Month-Yr.)
883	rhizome/genomic DNA	1, 2	*Halodule beaudettei*	(C. den Hartog) C. den Hartog	Shoal Grass	Corpus Christi, TX	27°39'08.41"N, 97°16'40.78"W	Jan-09
5769	rhizome/RNA extraction→cDNA	1	*Halodule beaudettei*	(C. den Hartog) C. den Hartog	Shoal Grass	Corpus Christi, TX	27°44'15.76"N, 97°08'18.22W	Aug-10
5770	rhizome/RNA extraction→cDNA	3	*Halodule beaudettei*	(C. den Hartog) C. den Hartog	Shoal Grass	Corpus Christi, TX	27°44'15.76"N, 97°08'18.22W	Aug-10
5771	rhizome/RNA extraction→cDNA	4	*Halodule beaudettei*	(C. den Hartog) C. den Hartog	Shoal Grass	Corpus Christi, TX	27°44'15.76"N, 97°08'18.22W	Aug-10
5772	rhizome/RNA extraction→cDNA	5	*Halodule beaudettei*	(C. den Hartog) C. den Hartog	Shoal Grass	Corpus Christi, TX	27°44'15.76"N, 97°08'18.22W	Aug-10
5773	rhizome/RNA extraction→cDNA	6	*Halodule beaudettei*	(C. den Hartog) C. den Hartog	Shoal Grass	Corpus Christi, TX	27°44'15.76"N, 97°08'18.22W	Aug-10
5774	rhizome/RNA extraction→cDNA	7	*Halodule beaudettei*	(C. den Hartog) C. den Hartog	Shoal Grass	Corpus Christi, TX	27°44'15.76"N, 97°08'18.22W	Aug-10
5775	rhizome/RNA extraction→cDNA	8	*Halodule beaudettei*	(C. den Hartog) C. den Hartog	Shoal Grass	Corpus Christi, TX	27°44'15.76"N, 97°08'18.22W	Aug-10

le XI. *GAPDH cDNA sequence descriptions for* H. beaudettei *cont…*

Bank ession mber	Source	Clone ID	Organism	Species Authors	Common Name	Collection Locality	Coordinates	Collection Da (Month-Yr.)
5776	rhizome/RNA extraction→cDNA	9	*Halodule beaudettei*	(C. den Hartog) C. den Hartog	Shoal Grass	Corpus Christi, TX	27°44'15.76"N, 97°08'18.22W	Aug-10
5777	rhizome/RNA extraction→cDNA	10	*Halodule beaudettei*	(C. den Hartog) C. den Hartog	Shoal Grass	Corpus Christi, TX	27°44'15.76"N, 97°08'18.22W	Aug-10