UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
CENTRO DE BIOTECNOLOGIA
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA CELULAR E MOLECULAR

PERFIL TRANSCRICIONAL DE GENES RELACIONADOS À DORMÊNCIA EM GEMAS DE MACIEIRA

Dissertação de Mestrado

Vítor da Silveira Falavigna

Porto Alegre, 2012

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Dissertação submetida ao Programa de Pós-graduação em Biologia Celular e Molecular do Centro de Biotecnologia da Universidade Federal do Rio Grande do Sul como requisito parcial para obtenção do Grau de Mestre.

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Porto Alegre, 2012

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Este trabalho foi desenvolvido no Laboratório de Genética Molecular Vegetal, no Centro Técnico da Embrapa Uva e Vinho. Recebeu auxílio financeiro da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), da Financiadora de Estudos e Projetos (FINEP) e da Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA).

AGRADECIMENTOS

A Deus, primeiramente, pela oportunidade de vida.

Aos meus pais, Dalva e Getulio, que apoiaram minhas decisões sempre incentivando os meus estudos e fornecendo todas as condições para eu alcançar o sucesso.

À minha irmã, Paula, que apesar da distância sempre demonstrou entusiasmo com o meu trabalho, fornecendo palavras de apoio e incentivo.

Ao Dr. Luís Fernando Revers, pesquisador da EMBRAPA e responsável pelo Laboratório de Genética Molecular Vegetal, por sua orientação, paciência e entusiasmo ao longo desses anos, principalmente pelo apoio e os conhecimentos compartilhados.

Ao Prof. Dr. Giancarlo Pasquali, por sua orientação, especialmente pela confiança e entusiasmo com o projeto, sempre apoiando e transmitindo conhecimentos.

À Profa. Dra. Márcia Margis-Pinheiro, por sua orientação, em especial pelas produtivas discussões sempre agregando conhecimento e ajudando a manter o foco durante a realização deste trabalho.

À Universidade Federal do Rio Grande do Sul, pela oportunidade de estudo em uma universidade pública e de qualidade.

À EMBRAPA Uva e Vinho, por permitir a utilização da sua estrutura e fornecer recursos para a realização deste projeto.

Ao CAPES, pela concessão de bolsa de Mestrado.

Ao pessoal do Laboratório de Genética Molecular Vegetal, Diogo, Vanessa, Yohanna, Roberta, Jaiana e Andriele, pela amizade e carinho, em especial ao Doutor Diogo Denardi Porto, pelo tempo empregado em discussões sobre o rumo deste – e outros – trabalhos.

Ao PPGBCM e ao CBiot, pela qualidade de ensino, especialmente a Janette e Rogério, minha Comissão de Acompanhamento, pela atenção despendida, e a Sílvia e Luciano, pela agilidade e prontidão.

Aos Professores da Banca Examinadora, pela disponibilidade, pelas críticas e sugestões.

À minha namorada Jana, pelo amor, carinho, incentivo e (muita!) paciência durante a realização deste trabalho, sempre me motivando e colocando meus pés no chão.

A todos aqueles que de forma direta ou indireta tenham contribuído para a realização deste trabalho.

SUMÁRIO

LISTA DE ABREVIATURAS, SÍMBOLOS E UNIDADES

ABA – ácido abscísico (do inglês, *abscisic acid*)

ABRE – elemento responsivos ao ABA (do inglês, *ABA Responsive Element*)

ADH – álcool desidrogenase

AGL24 – *AGAMOUS-LIKE 24*

AP1 – *APETALA1*

AP2 – *APETALA2*

ARP6 – proteína relaciona à actina 6 (do inglês, actin-related protein 6)

BLAST – do inglês, *Basic Local Alignment Sequence Tool*

bZIP FD – domínio básico zíper de leucina FD (do inglês, *Basic Leucine Zipper Domain FD*)

CAMTA – ativador da transcrição ligada à calmodulina (do inglês, *Calmodulin Binding Transcription Activator*)

CBFs – fatores de ligação à repetição C (do inglês, *C-repeat Binding Factors*)

cDNA – DNA complementar (do inglês, *complementary DNA*)

CH – cianamida hidrogenada

CO – *CONSTANS*

COR – responsivo ao frio (do inglês, *Cold Responsive*)

CRT – do inglês, *C-repeat*

DAM – *MADS-box* associados à dormência (do inglês, *dormancy-associated MADS-box*)

DHN – desidrinas (do inglês, *dehydrin*)

DNA – ácido desoxirribonucleico (do inglês, *deoxyribonucleic acid*)

DNase – desoxirribonuclease (do inglês, *deoxyribonuclease*)

DRE – elementos responsivos à seca (do inglês, *Drought Responsive Element*)

DREBs – proteínas de ligação a elementos responsivos à desidratação (do inglês, *Dehydration-Responsive Element-Binding proteins*)

EF – fator de alongamento (do inglês, *elongation factor*)

ESK1 – *ESKIMO1*

ESTs – etiquetas ou marcas de sequências expressas (do inglês, *Expressed Sequence Tags*)

FAO – Organização das Nações Unidas para Agricultura e Alimentação (do inglês, *Food and Agriculture Organization of the United Nations*)

FLC – *FLOWERING LOCUS C*

LFY – *LEAFY*

FT – *FLOWERING LOCUS T*

GA – ácido giberélico (do inglês, *gibberellic acid*)

GAST – transcrito estimulado por GA (do inglês, *GA Stimulated Transcript*)

GO – ontologia gênica (do inglês, *Gene Ontology*)

GoLS – galatinol sintase (do inglês, *galactinol synthase*)

GRAS – GAI, RGA e SCR (que, do inglês, designam respectivamente os *loci* "insensível à giberelina" ou *Gibberellin-Insensitive*; "repressor de ga1-3" ou *repressor of ga1-3*; e SCARECROW)

ICE1 – indutor de expressão de CBF (do inglês, *Inducer of CBF Expression 1*)

ICEr2 – do ingles, I*nduction of CBF Expression Region 2*

LOS1 – baixa expressão de genes osmoticamente responsivos (do inglês, *Low expression of Osmotically responsive genes 1*)

LTI65 – induzido por baixa temperatura 65 (do inglês, *low temperature induced 65*)

mRNA – RNA mensageiro (do inglês, *messenger RNA*)

miRNA – micro RNA

MYC/MYB – mielocitomatose/mieloblastose oncogene (do inglês, *myelocytomatosis*/*myeloblastosis oncogene*)

NAC – NAM, ATAF1/2, CUC2 (que, do inglês, designam respectivamente os *loci* "sem meristema apical" ou *No Apical Meristem*; ATAF1/2; "cotilédone em forma de taça 2" ou *Cup-shaped cotyledon 2*)

pb – pares de bases

PCR – reação em cadeia da DNA polimerase (do inglês, *polymerase chain reaction*)

PDC – piruvato descarboxilase

PHYA – fitocromo A (do inglês *PHYTOCROME A*)

PLACE – do ingles, *Plant Cis-acting Regulatory DNA Elements*

PRC2 – complexo repressivo do policombo (do inglês, *Polycomb Repressive Complex 2*)

RAP2.12 – relacionado ao APETALA2.12 (do inglês, *Related to APETALA2.12*)

RFO – oligossacarídeos da família da rafinose (do inglês, *Raffinose Family Oligosaccharides*)

RNA – ácido ribonucléico (do inglês, *ribonucleic acid*)

RT-qPCR – PCR quantitativa precedida de transcrição reversa (do inglês, *reverse transcription-quantitative PCR*)

SOC1 – supressor da superexpressão de CO1 (do inglês, *Suppressor of Overexpression of CONSTANS1*)

SCL – *SCARECROW-like*

SSH – hibridização supressiva subtrativa (do inglês, *Suppression Subtractive Hybridization*)

SVP – fase vegetativa curta (do inglês, *Short Vegetative Phase*)

T6PS – trealose 6-fosfato sintase (do inglês, *trehalose 6-phosphate synthase*)

UFH – unidades de frio hibernal

VIN3 – insensível a vernalização 3 (do inglês, *Vernalization Insensitive 3*)

ZAT12 – transportador de zinco 12 (do inglês, *zinc transporter 12*)

RESUMO

A macieira (*Malus* x *domestica* Borkh.) é uma frutífera de clima temperado que possui grande importância econômica mundialmente, sendo sua produtividade intimamente relacionada à saída do processo de dormência hibernal. Este processo pode ser definido como a incapacidade da planta iniciar o crescimento meristemático mesmo sob condições favoráveis e os mecanismos de controle molecular da dormência em macieiras ainda são pouco compreendidos. O objetivo do presente trabalho foi investigar o perfil gênico diferencial entre cultivares de macieiras contrastantes para requerimento de frio. As cultivares selecionadas foram Gala e sua mutante espontânea Castel Gala, as quais apresentam alto e baixo requerimento de frio, respectivamente. A técnica de hibridização supressiva subtrativa (SSH) permitiu a identificação de 28 genes candidatos à regulação da dormência. Análises de RT-qPCR foram realizadas visando a validação da expressão diferencial dos genes selecionados, assim como caracterizá-los transcricionalmente em três cultivares distintas durante um ciclo de crescimento e de dormência. Dos 28 genes candidatos, 17 apresentaram o mesmo perfil diferencial identificado por SSH. Um acúmulo sazonal de transcritos durante o inverno foi identificado para alguns genes e as cultivares de maior requerimento de frio apresentaram acúmulo de transcritos por mais tempo. Este perfil permitiu-nos sugerir que estes genes podem estar atuando na regulação dos processos de dormência e de aclimatação ao frio. Dos 17 genes validados, aqueles codificadores de proteínas DAM, desidrinas, GAST1, LTI65, NAC, histonas variantes H2A.Z e RAP2.12 apresentaram os maiores contrastes transcricionais entre as cultivares analisadas durante o inverno e constituem-se como fortes candidatos a participantes do processo de progressão da dormência em macieiras. Finalmente, a família de genes codificadores de desidrinas de macieira teve seus membros identificados e caracterizados transcricionalmente. Análises *in silico* permitiram a identificação de oito modelos gênicos preditos de desidrinas no genoma de macieira. As cadeias peptídicas deduzidas foram classificadas conforme a presença dos segmentos conservados Y_nSK_n. Um perfil sazonal de regulação da expressão foi identificado, com a presença de um pico de acúmulo de transcritos durante o inverno, o que sugere a presença de um mecanismo similar de regulação entre genes de desidrinas de macieira.

ABSTRACT

Apple tree (*Malus* x *domestica* Borkh.) is a temperate fruit crop of great economic importance worldwide and its productivity is related with the release from a bud dormancy process. This process is defined as the plant inability to initiate growth from meristems under favorable conditions and molecular information about dormancy control in apple trees is limited. The aim of the present work was to investigate the differential gene expression profiles between apple tree cultivars contrasting in chilling requirement for breaking dormancy. The selected apple cultivars were Gala and its derived bud sport Castel Gala, which displays high and low chilling requirement, respectively. A suppression subtractive hybridization (SSH) assay yielded 28 candidate genes putatively associated to dormancy cycling. RT-qPCR analyses were performed in order to validate the differential expression profiles and also to transcriptionally characterize the selected genes in three distinct apple tree cultivars during a growth to dormancy cycle. Among the 28 candidate genes, 17 confirmed the differential expression profile predicted by SSH. A seasonal transcript accumulation during the winter was identified to some genes, with high chilling requirement cultivars presenting higher levels of transcripts. This profile allowed us to suggest that these genes may be acting on dormancy regulation and cold acclimation. Out of the 17 candidate genes, those coding for DAM, dehydrins, GAST1, LTI65, NAC, histone variants H2A.Z and RAP2.12 displayed major differences in gene expression between cultivars through the winter and are strong candidates to play key roles on dormancy progression in apple trees. Finally, we identified and transcriptionally characterized the dehydrin gene family in apple trees. *In silico* analyses allowed us to identify eight predicted gene models for dehydrins in the apple genome. Deduced polypeptides were classified according to the presence of the conserved Y_nSK_n segments. A seasonal regulation of gene expression was observed, with higher transcript accumulation during the winter. This data suggests that a similar mechanism of transcript regulation is acting through the apple dehydrin genes.

1 INTRODUÇÃO

O processo de dormência de gemas em plantas perenes é um mecanismo de proteção a condições ambientais adversas tais como a exposição a baixas temperaturas e a alteração do fotoperíodo, estresses que podem acarretar a morte de tecidos metabolicamente ativos (HORVATH *et al.*, 2003; CAMPOY *et al.*, 2011). Tal processo é geneticamente controlado e sua superação ocorrerá associada à exposição das plantas a temperaturas de frio e fotoperíodo adequados a cada espécie.

Frutíferas de clima temperado possuem grande importância econômica mundial e sua produtividade está intimamente relacionada à dormência. Por consequência, a produção destas frutas em climas quentes apresenta dificuldades para a obtenção de altos rendimentos. A utilização de agentes químicos ou meios físicos visando a quebra da dormência de gemas é uma alternativa para a obtenção de sucesso em tais produções (PALLADINI & PETRI, 1999; DENARDI & SECCON, 2005; CAMPOY *et al.*, 2011).

As previsões de mudanças climáticas globais sugerem elevação das temperaturas médias anuais e invernos amenos, chamando a atenção às dificuldades que a agricultura enfrentará em médio e longo prazos (ARORA *et al.*, 2003; FERREIRA, 2009; CAMPOY *et al.*, 2011). Assim, o desenvolvimento de frutíferas com menor requerimento de frio constitui uma alternativa interessante visando à redução dos custos de produção associados à aquisição de agentes químicos, uma estratégia vantajosa do ponto de vista da sustentabilidade ambiental, em paralelo ao estabelecimento de uma maior uniformidade de brotação nas plantas cultivadas.

Os mecanismos moleculares que regulam a saída da dormência de gemas ainda são pouco compreendidos. Por outro lado, diversos genes já foram identificados devido à sua participação no processo de indução da dormência, especialmente quando induzido por exposição a dias curtos. Estudos realizados em álamo alpino e pêssego demonstraram que a utilização de mutantes divergentes quanto ao processo de dormência, sejam eles naturais ou obtidos por transgenia, é uma estratégia que pode ser empregada para a identificação de genes reguladores deste processo (BÖHLENIUS *et al.*, 2006; BIELENBERG *et al.*, 2008).

A macieira (*Malus* x *domestica* Borkh.) é um exemplo de planta regulada por fatores ambientais como a progressão da dormência dependente de frio (HEIDE & PRESTRUD,

2005). Contudo, distintos tempos de exposição a temperaturas de frio são necessários para superar o processo de dormência em diferentes cultivares de uma mesma espécie (JACKSON, 2003). Tal fato é observado entre a cultivar Gala Standard – que apresenta médio requerimento de frio – e alguns de seus mutantes naturais como a cultivar Castel Gala – que apresenta baixo requerimento de frio (PALLADINI & PETRI, 1999; DENARDI & SECCON, 2005). A caracterização da expressão gênica diferencial entre estas cultivares poderá permitir a identificação dos genes associados a um padrão de menor requerimento de frio, os quais poderão ser úteis para o desenvolvimento de cultivares geneticamente modificadas mais adaptadas às atuais condições ambientais de produção da região sul do Brasil.

1.1 DORMÊNCIA DE GEMAS E TRANSIÇÃO FLORAL EM PLANTAS

A identificação e a compreensão dos processos que regem o desenvolvimento de espécies perenes, em especial a mudança da fase juvenil à reprodutiva, a progressão da dormência e a floração, são essenciais para a obtenção de culturas comerciais melhor adaptadas ao seu cenário de cultivo regional (HORVATH *et al.*, 2003; JACKSON, 2003). Plantas perenes podem responder a condições ambientais sazonais com o estabelecimento de um período de dormência, o qual pode ser definido como a incapacidade de iniciar o crescimento meristemático sob condições favoráveis (ROHDE & BHALERAO, 2007).

Em resposta a diferentes fatores, três tipos de dormência em gemas foram previamente descritos: (i) paradormência – inibição do crescimento provocada por outro órgão da planta; (ii) endodormência – dormência regulada por sinais internos das gemas; e (iii) ecodormência – dormência devido a condições ambientais temporariamente desfavoráveis (LANG, 1987). Gemas de plantas perenes podem integrar os diferentes tipos de dormência e estar sob a ação de um ou mais sinais regulatórios simultaneamente, tais como resposta ao fotoperíodo e ao frio (FAUST *et al.*, 1997; HORVATH *et al.*, 2003). Neste sentido, as gemas atuam como estruturas receptoras desses sinais na planta, sendo que cada gema endodormente pode ser considerada como uma unidade independente. Estudos em pessegueiro demonstraram que não ocorre comunicação entre gemas endodormentes de um mesmo nó. Assim, uma determinada gema não é capaz de induzir a quebra da dormência em

outra gema da mesma planta (RAGEAU *et al.*, 2010). Na presente Dissertação, o foco principal foi o estudo do fenômeno de endodormência, o qual será chamado de dormência a partir deste ponto.

Diferentes fatores ambientais, fisiológicos e moleculares já tiveram seu papel parcialmente caracterizado no processo de dormência. Contudo, a hipótese de que apenas um sinal atua diretamente nas gemas, sendo responsável pela ativação e manutenção da dormência, é improvável (JACKSON, 2003; CHAO *et al.*, 2007). A interação entre esses sinais regulatórios ainda é pouco conhecida, apesar dos recentes avanços conquistados na área.

1.1.1 Mecanismos fisiológicos da dormência

O estabelecimento do processo de dormência consiste na paralisação do crescimento vegetativo, na formação da gema apical e no início da senescência das folhas. Fatores ambientais como a sequência de dias mais curtos e a exposição a baixas temperaturas são sinais que induzem a planta a entrar no processo de dormência (HORVATH *et al.*, 2003; JACKSON, 2003; ROHDE & BHALERAO, 2007). Uma vez que o crescimento vegetativo cessa, as células meristemáticas ficam inabilitadas de responder a sinais de promoção de crescimento (ROHDE & BHALERAO, 2007).

A progressão da dormência está relacionada à exposição da planta ao frio prolongado. Cada espécie vegetal possuirá um requerimento de frio hibernal específico da sua região de origem. Porém, evolutivamente, poderá se adaptar a diferentes regiões (JACKSON, 2003). Diversos modelos empíricos foram desenvolvidos visando a estimativa do requerimento de frio de cada espécie, sendo a forma de registro destes as unidades de frio hibernal (UFH). Os modelos “Utah Modificado” e “Carolina do Norte Modificado” estão baseados na acumulação de UFH conforme uma tabela predefinida, onde a exposição à determinada temperatura por uma hora equivale a certa quantidade de UFH (JACKSON, 2003; BOTELHO *et al.*, 2006). Uma vez satisfeita a necessidade de frio, a planta estará apta a reiniciar o ciclo vegetativo e reprodutivo quando houver condições ambientais favoráveis (JACKSON, 2003; ROHDE & BHALERAO, 2007).

O ácido giberélico (GA, do inglês, *gibberellic acid*) é um hormônio vegetal responsável por controlar o alongamento e a diferenciação celular. Uma diminuição na concentração de GA foi observada em plantas expostas a dias curtos, ocasionando a paralisação do crescimento vegetativo e da divisão celular nos meristemas subapicais. Tal observação é sustentada pela regulação negativa imposta ao gene *oxidase GA-20* em folhas, o qual codifica uma enzima chave na síntese de GA (ALLONA *et al.*, 2008; van der SHOOT & RINNE, 2011).

A aplicação exógena de GA é capaz de quebrar a dormência de gemas em substituição ao frio. Assim, sugere-se que o frio seja capaz de estimular a biossíntese de GA, além de ativar genes de sua síntese no ápice da gema por meio de modificações no padrão de metilação destes genes. Contudo, a regulação da rota de síntese de GA parece possuir maior importância na dormência do que o nível de GA presente nos tecidos (van der SHOOT & RINNE, 2011).

Ácido abscísico (ABA, do inglês, *abscisic acid*) é um fitohormônio envolvido na resposta a estresses abióticos, na maturação de sementes e na paralisação do crescimento. A sua interação com íons cálcio em resposta a estresses ambientais envolve a indução de genes envolvidos na síntese e na transdução de sinal de ABA (ARORA *et al.*, 2003; van der SHOOT & RINNE, 2011). Contudo, o seu papel durante o processo de dormência de gemas é controverso (ROHDE & BHALERAO, 2007; ALLONA *et al.*, 2008). Embora o nível de ABA alcance um pico após três a quatro semanas de exposição a dias curtos, resultando na ativação de genes da rota de síntese e de transdução de sinal de ABA, nenhuma correlação foi comprovada entre a paralisação do crescimento vegetativo, a dormência de gemas e os níveis apicais de ABA em plantas perenes. O seu envolvimento na dormência parece estar mais relacionado ao controle do fotoperíodo para a aclimatação ao frio e a tolerância à desidratação (ARORA *et al.*, 2003; ALLONA *et al.*, 2008; van der SHOOT & RINNE, 2011).

Açúcares, além de fontes de energia, são moléculas sinalizadoras que regulam a expressão de diversos genes durante o desenvolvimento vegetal (RIOU-KHAMLICHI *et al.*, 2000). Algumas de suas rotas de sinalização envolvem a transição de gemas vegetativas da paradormência para a endodormência. Especula-se que alguns açúcares, tais como sacarose, amido, galactinol e rafinose, possuam um papel importante na regulação da dormência de gemas por meio da sua interação com fitormônios, como o ABA (CHAO *et al.*, 2007; RUTTINK *et al.*, 2007).

1.1.2 Mecanismos moleculares da dormência

A dormência de gemas em plantas de clima temperado é um fenômeno bem estudado do ponto de vista fisiológico e de suas interações com fatores ambientais. Entretanto, os aspectos moleculares e os componentes genéticos envolvidos nas rotas de sinalização e regulação da dormência ainda são poucos caracterizados (HORVATH *et al.*, 2003; HORVATH, 2009). Assim, a sobreposição dos sinais que regulam os processos de dormência, floração e percepção de temperatura fazem estes fenômenos interdependentes, sendo necessário que sejam estudados e compreendidos conjuntamente.

1.1.2.1 Percepção ao frio

As variações que ocorrem na temperatura ao longo das estações do ano possuem papel regulatório em diversos estádios do desenvolvimento vegetal, uma vez que a planta necessita perceber tais oscilações para responder satisfatoriamente (PENFIELD, 2007). O processo de dormência é um mecanismo de sobrevivência da planta sob condições adversas tais como os invernos em regiões de climas frios e temperados, necessitando que a indução deste fenômeno ocorra antes da chegada de temperaturas extremas de frio (CAMPOY *et al.*, 2011).

As etapas de indução e superação da dormência em climas temperados são primariamente reguladas por baixas temperaturas e alterações no comprimento do dia. Contudo, a extensão na qual estes sinais ambientais regulam tal processo e sua interação com rotas de sinalização varia grandemente entre espécies (CHAO *et al.*, 2007). Pereiras e macieiras, por exemplo, possuem o estabelecimento e a indução da dormência exclusivamente controlada por baixas temperaturas, independentemente de alterações no fotoperíodo (HEIDE & PRESTRUD, 2005). Especula-se que este tipo de resposta seja um padrão entre plantas originárias de regiões onde a temperatura marca as estações do ano com maior precisão do que a qualidade da luz, possuindo mecanismos evolutivamente mais direcionados à sensibilidade e à percepção de temperaturas para a sincronização da sua fenologia com o ambiente (CAMPOY *et al.*, 2011).

O fenômeno de aclimatação ao frio – processo no qual após prévia exposição a baixas temperaturas as plantas ganham tolerância a temperaturas extremas de frio – exemplifica a importância da percepção do frio por parte das plantas, uma vez que antecede a paralisação do crescimento vegetal (PENFIELD, 2007; TANINO *et al.*, 2010). Diversos mecanismos moleculares de percepção ao frio já foram elucidados em plantas, apesar de muitas rotas de sinalização ainda necessitarem de maiores estudos (CAMPOY *et al.*, 2011).

As proteínas CBFs (fatores de ligação à repetição C, do inglês, *C-repeat Binding Factors*), também conhecidas como DREBs (proteínas de ligação a elementos responsivos à desidratação, do inglês, *Dehydration-Responsive Element-Binding proteins*), possuem função central na indução de genes de aclimatação ao frio. Estes fatores de transcrição são induzidos de três a seis horas após a exposição ao frio, ativando a expressão de genes *COR* (responsivo ao frio, do inglês, *Cold Responsive*) e conferindo tolerância ao frio (CHINNUSAMY *et al.*, 2007; KURBIDAEVA & NOVOKRESHCHENOVA, 2011).

O fator de transcrição ICE1 (indutor de expressão de CBF, do inglês, *Inducer of CBF Expression 1*) possui forma ativa somente sob baixas temperaturas por meio de modificações postraducionais induzidas pelo frio. Em *Arabidopsis*, o gene *ICE1* é constitutivamente expresso, sendo um dos primeiros integrantes da cascata de sinalização por frio. Uma vez ativado, a sua proteína forma dímeros capazes de interagir com o DNA e induzir a expressão dos genes *CBF*s (CHINNUSAMY *et al.*, 2007; KURBIDAEVA & NOVOKRESHCHENOVA, 2011).

A família de fatores de transcrição CAMTA (ativador da transcrição ligada à calmodulina, do inglês, *Calmodulin Binding Transcription Activator*) atua na sinalização de íons cálcio por meio de interação com calmodulinas. Em plantas, íons cálcio são mensageiros secundários que atuam em cascatas de transdução de sinal em diversos processos de crescimento e desenvolvimento, incluindo respostas a vários estímulos ambientais (PANG *et al.*, 2007). O frio é um dos estímulos responsáveis pelo aumento da concentração destes íons no citoplasma, iniciando uma cascata de transdução de sinal. A interação entre calmodulinas e cálcio regula a atividade das proteínas CAMTA, as quais são capazes de interagir com a região promotora do gene *CBF2* ativando-o, com impacto na aclimatação ao frio (DOHERTY *et al.*, 2009; KURBIDAEVA & NOVOKRESHCHENOVA, 2011).

As *DHN*s (desidrinas, do inglês, *dehydrins*) integram o conjunto de genes *COR* e suas proteínas são sintetizadas durante situações de desidratação celular, o que ocorre durante estágios de desenvolvimento ou em resposta a estímulos ambientais, tais como baixas

temperaturas, perda de água ou alta salinidade (KURBIDAEVA & NOVOKRESHCHENOVA, 2011). Estudos em diferentes espécies vegetais lenhosas demonstraram que o acúmulo de algumas destas proteínas inicia durante o outono e alcança seu maior nível no inverno, coincidindo com a máxima tolerância a temperaturas extremas de frio (FERNANDEZ *et al.*, 2012). O acúmulo de DHNs durante a aclimatação ao frio é importante para a proteção do citoplasma e de membranas lipídicas contra a perda de água, além de possuir atividade crioprotetora sobre diversas enzimas (KOSOVÁ *et al.*, 2007). As *DHNs* estão presentes em gemas expostas ao frio e, em geral, desaparecem quando o crescimento vegetativo é restabelecido (KEILIN *et al.*, 2007).

Outras rotas de aclimatação ao frio independentes dos genes *CBFs* já foram descritas. Contudo, a grande maioria desses mecanismos ainda é pouco conhecida. Os genes *ESK1* (ESKIMO1), *MYC*/*MYB* (mielocitomatose/mieloblastose oncogene, do inglês, *MYeloCytomatosis*/*MYeloBlastosis oncogene*), *NAC* (NAM, ATAF1/2, CUC2 que, do inglês, designam respectivamente os *loci* "sem meristema apical" ou *No Apical Meristem*; ATAF1/2; "cotilédone em forma de taça 2" ou *Cup-shaped cotyledon 2*), *ZAT12* (transportador de zinco 12, do inglês, *zinc transporter 12*), entre outros, integram rotas alternativas de percepção ao frio, as quais podem ser dependentes ou independentes de ABA (CHINNUSAMY *et al.*, 2007; KURBIDAEVA & NOVOKRESHCHENOVA, 2011).

O gene *ESK1* codifica uma proteína que atua na rota dependente de ABA e possui função regulatória negativa na percepção ao frio. Em Arabidopsis, este integra uma família gênica de 45 proteínas, cuja grande maioria ainda não foi caracterizada. O mecanismo no qual ESK1 regula a tolerância a temperaturas extremas de frio ainda não foi elucidado (XIN & BROWSE, 1998).

Os fatores de transcrição da família NAC são exclusivos de plantas e possuem mais de 100 membros, dos quais apenas uma pequena quantidade teve sua função caracterizada (OLSEN *et al.*, 2005). Alguns de seus membros já foram descritos por integrar rotas de respostas à desidratação e ao estresse salino independente de ABA, sendo também demonstrada, em *Arabidopsis*, a sua participação no aumento da tolerância ao frio (KURBIDAEVA & NOVOKRESHCHENOVA, 2011).

O gene *LOS1* (baixa expressão de genes osmoticamente responsivos, do inglês, *Low expression of Osmotically responsive genes 1*) codifica um fator de alongamento (EF2) da cadeia peptídica durante a tradução. Em *Arabidopsis*, o processo de tradução de proteínas em

baixas temperaturas é dependente de LOS1, uma vez que a falta desta proteína resulta na diminuição drástica desse processo no frio (GUO *et al.*, 2002).

Oligossacarídeos da família da rafinose (RFO, do inglês, *Raffinose Family Oligosaccharides*) foram descritos como boas moléculas de reserva de carbono devido à sua atividade não redutora, acumulando-se em grandes quantidades na célula sem afetar processos metabólicos primários (UNDA *et al.*, 2012). Tais carboidratos foram descritos inicialmente pelo seu papel na dessecação de sementes e posteriormente na tolerância ao estresse provocado pelo frio, salinidade e seca (TAJI *et al.*, 2002). A enzima galatinol sintase (GoLS, do inglês, *galactinol synthase*) é responsável pela primeira etapa da reação de síntese de RFOs. Em *Arabidopsis*, a regulação de três homólogos de *GoLS* ocorre em resposta à exposição a diferentes tipos de estresses de desidratação, sendo um desses homólogos regulado exclusivamente pelo frio (TAJI *et al.*, 2002). Estudos recentes em álamo demonstraram que os genes da família *GoLS* são regulados diferentemente entre si ao longo do ano e que um dos homólogos teve sua expressão induzida durante o inverno (UNDA *et al.*, 2012).

O açúcar trealose é um dissacarídeo capaz de proteger moléculas biológicas por meio da absorção reversível de água em resposta a condições de estresse e dessecação, como exposição a altas ou baixas temperaturas. Contudo, o acúmulo deste carboidrato é comum apenas em plantas altamente tolerantes à dessecação, estando em quantidades irrisórias na grande parte do reino vegetal (PENNA, 2003). A descoberta de genes que regulam a síntese de trealose em *Arabidopsis*, como o codificador da trealose 6-fosfato sintase (T6PS, do inglês, *trehalose-6-phosphate synthase*), instigou a busca desta rota metabólica em diversas espécies vegetais. A produção da enzima T6PS, responsável pela primeira etapa do catabolismo de trealose, é de vital importância para a germinação de sementes de *Arabidopsis*, além de possuir um papel importante no crescimento vegetativo e na transição floral de plantas adultas (DIJKEN *et al.*, 2004).

A exposição ao frio possui diferentes efeitos sobre as plantas. Baixas temperaturas podem promover danos celulares irreversíveis caso a planta não passe por aclimatação ao frio. Contudo, o conhecimento dos mecanismos de resposta a este tipo de estresse ainda é limitado (KURBIDAEVA & NOVOKRESHCHENOVA, 2011). A percepção às mudanças de temperatura e a sua interação com outros sinais ambientais possui papel regulatório no controle de diferentes processos referentes ao desenvolvimento e a sobrevivência das plantas,

tais como a progressão da dormência e da floração (PENFIELD, 2008; AMASINO & MICHAELS, 2010).

1.1.2.2 Transição floral

O fenômeno de transição floral abrange todos os mecanismos necessários para que o meristema produza flores. O controle da floração envolve a ativação de genes homeóticos florais por meio da integração de sinais ambientais – como mudanças no fotoperíodo e na temperatura – e endógenos – tais como a sinalização por GA e rotas autônomas (WILKIE *et al.*, 2008; POSÉ *et al.*, 2012).

Em *Arabidopsis*, receptores de luz presentes nas folhas são capazes de perceber alterações no fotoperíodo, sendo que a presença de dias longos desencadeia a indução da floração (WILKIE *et al.*, 2008; AMASINO & MICHAELS, 2010). Fotorreceptores de vernalização para o relógio circadiano, tais como fitocromo A (PHYA, do inglês *PHYTOCROME A*) e criptocromo 1, regulam a transcrição do promotor floral *CONSTANS* (*CO*) de modo que o pico de transcritos ocorre ao entardecer em dias longos e à noite em dias curtos. Devido à proteína de *CO* ser degradada no escuro, ela somente irá se acumular durante dias longos para, assim, induzir a transcrição do integrador floral *FLOWERING LOCUS T* (FT) nas folhas (BÖHLENIUS *et al.*, 2006; WILKIE *et al.*, 2008; AMASINO & MICHAELS, 2010). A proteína de *FT* se encaixa na descrição de "florígeno", substância descrita em meados de 1930 por Chailakhyan (1937) como a molécula transportada pelo floema que é indutora de floração (BÖHLENIUS *et al.*, 2006). Esta proteína é transportada até o broto apical para formar um complexo com um fator de transcrição do tipo bZIP FD (domínio básico zíper de leucina FD, do inglês, *Basic Leucine Zipper Domain FD*). Apesar de sua presença no meristema ainda não ter sido comprovada, este complexo induz a floração pela ativação de genes de identidade meristemática como *APETALA1* (AP1), e genes de integração floral, como SOC1 (supressor da superexpressão de CONSTANS1, do inglês, *Suppressor of Overexpression of CONSTANS1*; AMASINO & MICHAELS, 2010; POSÉ *et al.*, 2012).

Uma das rotas de repressão de *FT* é mediada pela família de fatores de transcrição que contém o domínio AP2 (APETALA2), os quais são reguladores transcricionais de diversos

processos biológicos relacionados ao desenvolvimento vegetal, em especial a determinação de órgãos florais. O gene *AP2*, em *Arabidopsis*, foi descrito como repressor da floração e do desenvolvimento floral, por meio da inibição da transcrição de *SOC1* e de *miRNA172*, além de atuar na indução da transcrição de outros repressores florais da sua mesma família (YANT *et al*., 2010). O micro RNA *miRNA172* regula a expressão de genes inibidores de florescimento tais como aqueles da família *AP2*, incluindo o próprio gene *AP2*. O aumento da presença deste miRNA resulta na diminuição de transcritos dos genes repressores florais, resultando na promoção do florescimento (AUKERMAN & SAKAI, 2003).

Os fatores de transcrição da família *MADS-box* AGL24 (AGAMOUS-LIKE 24) e SVP (fase vegetativa curta, do inglês, *Short Vegetative Phase*) possuem alta similaridade de sequência. Entretanto, desempenham funções antagônicas no processo de transição floral. Enquanto AGL24 é capaz de formar um complexo proteico com SOC1 e promover a expressão do gene de identidade meristemática *LEAFY* (*LFY*), a proteína SVP atua como repressor da floração, regulando rotas autônomas e de temperatura em conjunto com FLC (*FLOWERING LOCUS C*; HORVATH, 2009; POSÉ *et al.*, 2012).

A vernalização é um processo onde a exposição prolongada ao frio é capaz de tornar as plantas competentes para a floração (AMASINO & MICHAELS, 2010). Tal processo é um dos responsáveis pela repressão do gene *FLC*. Este gene codifica um fator de transcrição do tipo *MADS-box* que atua em rotas repressoras de floração. FLC, em nível transcricional, é capaz de reprimir genes-chave de transição floral tais como *FD*, *FT* e *SOC1* (WILKIE *et al.*, 2008; AMASINO & MICHAELS, 2010). As baixas temperaturas são responsáveis pela ativação da expressão do gene *VIN3* (insensível a vernalização 3, do inglês, *Vernalization Insensitive 3*), o qual codifica componentes do complexo PRC2 (complexo repressivo do policombo, do inglês, *Polycomb Repressive Complex 2*). Este complexo é responsável por atuar na remodelação da cromatina do gene *FLC* por meio da metilação da lisina-27 na histona H3 (HORVATH, 2009; AMASINO & MICHAELS, 2010). A cromatina é uma fibra composta por DNA e proteínas em unidades repetitivas de nucleossomos, octâmeros compostos de duas cópias de cada uma das quatro proteínas histonas (H2A, H2B, H3 e H4), além de cerca de 150 pares de bases (pb) de DNA ao seu redor. Esta estrutura serve, principalmente, para compactar o DNA no núcleo, mas também tem função de regulação da expressão gênica por expor ou indisponibilizar sequências de DNA às proteínas reguladoras e ao complexo de transcrição (DEAL & HENIKOFF, 2010). Outro regulador da expressão do gene *FLC* por meio de eventos de remodelação da cromatina é o complexo SWR1C. Uma de

suas subunidades proteicas, codificada pelo gene *ARP6* (proteína relaciona à actina 6, do inglês, actin-related protein 6), possui como função substituir a histona H2A pela sua variante termossensível H2A.Z. Esta variante é responsável por manter a região promotora de determinados genes em um estado relaxado, preparado para a transcrição, mas dependente de aumento de temperatura para que ocorra a sua ativação (DEAL & HENIKOFF, 2010; KUMAR & WIGGE, 2010).

A indução da floração durante dias curtos necessita que o hormônio GA seja produzido. Neste sistema, GA_4 é produzido nas folhas e transportado até o broto apical, induzindo a floração por meio da indução de *SOC1* e *LFY* (WILKIE *et al.*, 2008; HORVATH, 2009). Genes da família *GAST* (transcrito estimulado por GA, do inglês, *GA Stimulated Transcript*) foram inicialmente descritos em tomateiro, sendo descrito o aumento de sua transcrição por meio de aplicação de GA (SHI *et al.*, 1992). Em *Arabidopsis*, alguns dos membros da família *GAST* foram descritos por atuarem no desenvolvimento floral, na germinação de sementes, na sinalização de luz e na resistência ao estresse causado por calor (RUBINOVICH & WEISS, 2010).

Os fatores de transcrição GRAS (GAI, RGA e SCR que, do inglês, designam respectivamente os loci “insensível à giberelina” ou *Gibberellin-Insensitive*; “repressor de ga1-3” ou *repressor of ga1-3*; e *SCARECROW*) possuem envolvimento no crescimento e no desenvolvimento vegetal, na sinalização por GA e na transdução de sinal de luz. Entretanto, apesar de 33 genes preditos terem sido identificados no genoma de *Arabidopsis*, apenas dez destes tiveram sua função descrita na literatura (LEE *et al.*, 2008).

O hormônio ABA, em contrapartida, possui função antagonista ao GA, atuando como repressor da floração. A sinalização induzida por ABA e etileno, um hormônio relacionado ao estresse, altera a floração devido à sua interação com as proteínas DELLA, as quais atuam na repressão da sinalização de GAs (HORVATH, 2009). Uma das subfamílias dos fatores de transcrição GRAS é denominada DELLA, devido à presença do domínio proteico DELLA. Dois dos seus integrantes, RGA e GAI, foram descritos por atuarem na regulação negativa à sinalização do hormônio GA (TYLER *et al.*, 2004).

1.1.2.3 Dormência de gemas

A compreensão dos mecanismos moleculares que controlam o estabelecimento, a manutenção e a liberação do processo de dormência em gemas é de grande valia para a geração de plantas adaptadas a cada cenário de cultivo. A transição do ciclo de dormência deve ser finamente sincronizada com as variações climáticas sazonais. Sinais ambientais tais como temperatura, fotoperíodo, qualidade de luz ou seca auxiliam a planta a regular este processo de sincronização (CAMPOY *et al.*, 2011). Entretanto, uma vez que a descoberta de genes reguladores da dormência teve início apenas recentemente, a sua perfeita integração com estes sinais ambientais e com rotas fisiológicas já estabelecidas ainda não foi realizada.

Estudos conduzidos com o mutante *evergrowing* (de crescimento contínuo) de pessegueiro, o qual é incapaz de formar gemas vegetativas terminais em resposta a condições indutoras de dormência, permitiram a identificação de uma mutação genômica onde se descobriu uma família de fatores de transcrição envolvida na regulação deste processo. Este mutante possui deleção de um segmento cromossômico contendo seis genes organizados em *tandem*, os quais apresentam alta similaridade ao grupo StMADS11, o qual também apresenta os genes *SVP* e *AGL14* de *Arabidopsis*. Os genes de pessegueiro foram descritos como fatores de transcrição DAM (*MADS-box* associados à dormência, do inglês, *dormancy-associated MADS-box*) e são candidatos à regulação da interrupção do crescimento vegetativo e à formação da gema terminal (BIELENBERG *et al.*, 2008). Dois desses genes, *PpDAM5* e *PpDAM6*, possuem um perfil transcricional consistente ao de repressores do crescimento vegetativo, com o aumento de transcritos no início da dormência e o declínio durante o inverno (LI *et al.*, 2009). Genes codificadores de proteínas desta mesma família já foram descritos em damasco (SASAKI *et al.*, 2011), *kiwi* (WU *et al.*, 2012) e pereira (UBI *et al.*, 2010).

Em *Arabidopsis*, os genes *FT* e *CO* foram descritos como responsáveis pela indução da floração e percepção do fotoperíodo, respectivamente (AMASINO & MICHAELS, 2010). Contudo, existem evidências de que a indução do processo de dormência de gemas por dias curtos é mediada pelo fitocromo e pelo módulo *FT/CO*. Plantas do gênero *Populus* superexpressando *FT* são incapazes de entrar em dormência quando expostas a dias curtos (BÖHLENIUS *et al.*, 2006).

O processo de progressão da dormência foi anteriormente relacionado com a sinalização por GA. A aplicação ectópica de GA na sua forma ativa é capaz de substituir a exposição ao frio (van der SCHOOT & RINNE, 2011). Recentemente, foi proposto que a liberação da dormência ocorre após o restabelecimento de conexões simplásticas por meio da degradação da calose do plasmodesma em um mecanismo induzido por GA (RINNE *et al.*, 2011). Em gemas, proteínas da família DELLA podem estar envolvidas no estabelecimento do processo de dormência, devido à ativação transcricional que ocorre nestes genes durante exposição a dias curtos. GA poderia regular estes genes durante o processo de superação da dormência, por meio da repressão da transcrição destes repressores do crescimento (van der SCHOOT & RINNE, 2011).

Estudos em videira relacionaram o evento de quebra da dormência com a sinalização por estresse oxidativo. A aplicação de agentes indutores da quebra de dormência de gemas, como cianamida hidrogenada (CH), leva ao desenvolvimento de estresse oxidativo, ativando uma cascata de transdução de sinal que, por sua vez, estimula a brotação (OR *et al.*, 2000). As enzimas piruvato descarboxilase (PDC) e álcool desidrogenase (ADH) estão envolvidas no processo de fermentação anaeróbica, o qual aparece normalmente em baixos níveis nas plantas, sendo induzidas apenas por distúrbios respiratórios. A aplicação de CH levou ao aumento da transcrição de *PDC* e *ADH*. Apesar da indução do metabolismo fermentativo ter sido de natureza transitória, uma vez que a indução destes genes parou cerca de quatro dias após a aplicação de CH, foi sugerido que este distúrbio respiratório possa ser uma transdução de sinal que culmina na saída da dormência (OR *et al.*, 2000).

Um modelo para o processo de regulação da dormência foi proposto por HORVATH (2009), onde os genes *FT/CENL1* e *DAM* desempenham papel central (Figura 1). Neste modelo, uma breve exposição ao frio induziria a expressão dos genes *DAM*, os quais atuariam como repressores do gene *FT*. A redução da expressão de *FT* seria responsável pela paralisação do crescimento vegetativo e a indução da dormência. A exposição prolongada ao frio reprimiria os genes *DAM* por meio da remodelação da cromatina, acarretando na superação da dormência (HORVATH, 2009).

Diferentemente da maioria das plantas perenes, algumas espécies da família das Rosáceas, incluindo a macieira e a pereira, não possuem o fotoperíodo como principal regulador do processo de indução e superação da dormência e, sim, as baixas temperaturas (HEIDE & PRESTRUD, 2005; HORVATH, 2009).

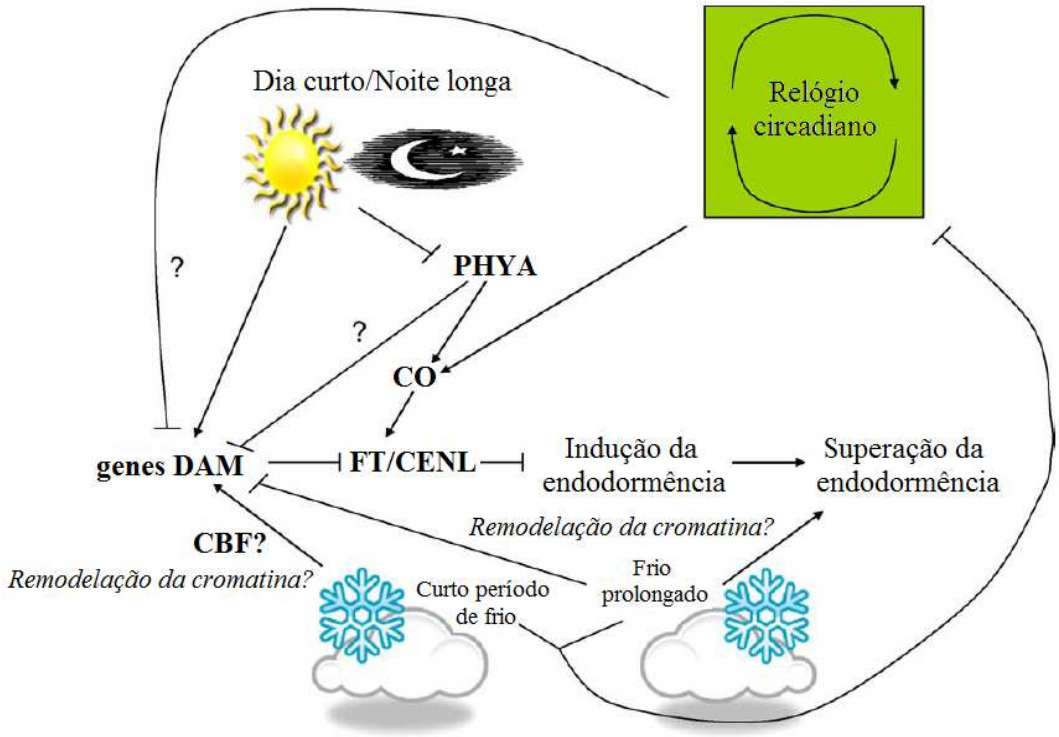

Figura 1. Modelo de indução da dormência. Curtos períodos de frio induzem os genes *DAM* por meio da ação dos CBFs e possivelmente por eventos de remodelação da cromatina. Alternativamente, a alteração do relógio circadiano devido à exposição a dias curtos reprime a expressão de *CO*, um indutor do gene *FT*, e induz a expressão dos genes *DAM*. A cascata de sinalização mediada por PHYA também é importante por integrar os sinais do relógio circadiano. A acumulação das proteínas DAM é capaz de reprimir *FT*. A redução de FT acarreta na paralisação do crescimento e na indução da dormência de gemas. A prolongada exposição ao frio leva à repressão dos genes *DAM* por meio de alterações na cromatina, além de inibir a sinalização do relógio circadiano, levando à quebra da dormência. Adaptado de HORVATH (2009).

1.2 A CULTURA DA MACIEIRA

A macieira é uma das principais frutíferas cultivadas mundialmente, sendo a quarta mais importante economicamente, atrás apenas de espécies cítricas, videira e bananeira (TROGGIO et al., 2012). Membro integrante da família das Rosáceas, esta espécie de clima temperado apresenta queda de folhas no final do seu ciclo vegetativo com o estabelecimento da dormência. Mais de 10.000 diferentes cultivares de macieira já foram documentadas (TROGGIO et al., 2012), sendo que, no Brasil, as principais cultivares comerciais são Gala, Fuji e suas variantes, devido às suas propriedades organolépticas que agradam ao consumidor brasileiro. Entretanto, ambas cultivares possuem alto requerimento de frio para a quebra de dormência, isto é, 800 e 1040 UFH, respectivamente. A insuficiência de frio acumulado

durante o inverno é um dos principais limitantes na produção de frutíferas de clima temperado, por causar irregularidade na brotação das gemas floríferas e vegetativas. Ocorre, assim, a necessidade da utilização de tratamentos químicos e físicos para que haja a continuidade do ciclo vegetativo da planta, gerando um aumento nos custos de produção e de manejo (PALLADINI & PETRI, 1999; DENARDI & SECCON, 2005).

No ano de 2010, de acordo com a Organização das Nações Unidas para Agricultura e Alimentação (FAO, do inglês, *Food and Agriculture Organization of the United Nations*), foram produzidas 69,5 milhões de toneladas de maçã, sendo que o Brasil ocupou o nono lugar na produção mundial com quase 1,3 milhões de toneladas. Entretanto, foi apenas a partir do ano de 1999 que o país tornou-se exportador de maçãs, fruto de investimentos realizados nos últimos 30 anos e que fizeram a produção aumentar em mais de 6.000% (FERREIRA, 2009). Analisando-se as previsões climáticas globais que sugerem um incremento de 1 a 2,5°C na temperatura média anual até o ano de 2050 na Região Sul do Brasil, a qual é responsável por mais de 90% da produção nacional de maçãs, destacam-se as dificuldades que surgirão para o aumento da produção e da qualidade das safras (ARORA *et al.*, 2003; FERREIRA, 2009; CAMPOY *et al.*, 2011). A redução no acúmulo de horas de frio necessárias para o controle da progressão da dormência surge como o principal fator limitante da produção (FERREIRA, 2009). A utilização de cultivares com baixa exigência de frio, tais como a cultivar Castel Gala, é uma interessante alternativa para contornar tal situação.

A cultivar Castel Gala é caracterizada por apresentar baixo requerimento de frio – aproximadamente 400 UFH – quando comparada a cultivares comerciais como Gala e Fuji. Esta nova cultivar surgiu em 1999 na cidade de Monte Castelo, SC, oriunda de uma mutação espontânea de gemas de um ramo lateral de ‘Gala’, e possuindo frutos com características físico-químicas muito similares a esta (DENARDI & SECCON, 2005). Por apresentar menor requerimento de frio, ‘Castel Gala’ possui seu ciclo de brotação, floração e produção de maçãs antecipado em torno de 25 dias quando comparada à ‘Gala’. Uma boa adaptação climática é essencial para que haja a antecipação da brotação e do florescimento, ficando assim evidenciada a boa adaptação de ‘Castel Gala’ ao clima sul brasileiro (DENARDI & SECCON, 2005). Na Figura 2 está apresentada uma ilustração representativa do contraste de desenvolvimento temporal existente entre as cultivares Gala e sua mutante Castel Gala.

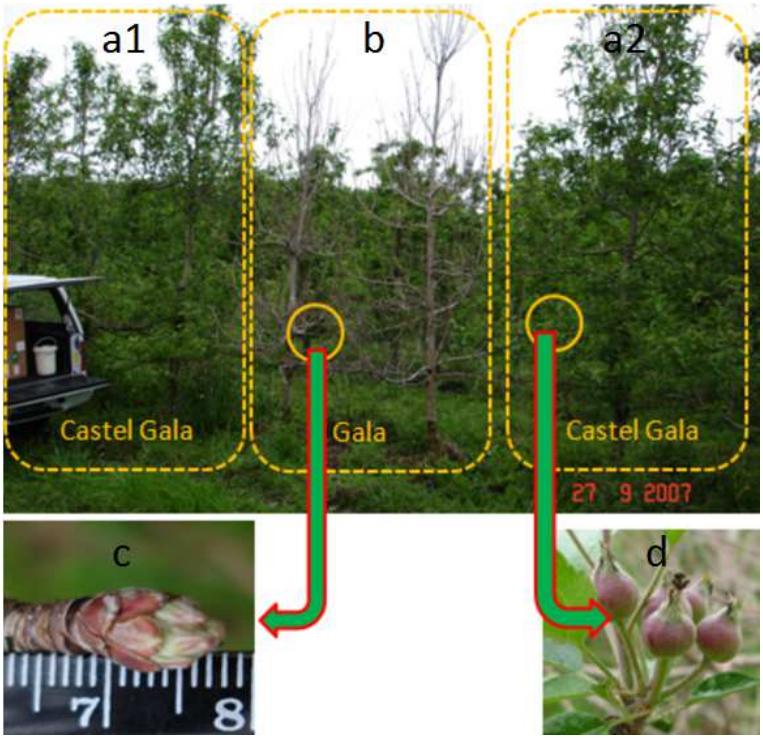

Figura 2. Contraste existente entre as cultivares Gala e Castel Gala de macieira. Enquanto 'Castel Gala' já iniciou o ciclo vegetativo e reprodutivo (em a1 e a2), 'Gala' ainda está sob efeito do processo de dormência (b). Destaca-se em "c" uma gema dormente de 'Gala', e em "d" mostram-se frutos de até um centímetro de diâmetro de 'Castel Gala' à mesma época.

Assim sendo, o contrastante requerimento de frio hibernal existente entre as referidas cultivares torna-se um modelo interessante para o estudo dos mecanismos moleculares envolvidos nos fenômenos de entrada e de saída da dormência de gemas em macieira. A utilização de metodologias que permitam a identificação de genes diferencialmente expressos é uma alternativa para explorar este modelo, visando contribuir para uma melhor elucidação dos mecanismos moleculares que regulam estes processos em plantas.

1.2.1 Expressão gênica diferencial explorando o modelo 'Gala' *vs* 'Castel Gala'

A técnica de Hibridização Supressiva Subtrativa (SSH, do inglês, *Suppression Subtractive Hybridization*) pode ser utilizada na amplificação seletiva de genes diferencialmente expressos, ao mesmo tempo em que suprime a amplificação de sequências

curtas de DNA (DIATCHENKO *et al.*, 1996). Uma série de estudos visando explorar a expressão gênica diferencial entre fenótipos contrastantes já foi realizada em macieiras. Para tal, a SSH vem sendo empregada com sucesso em macieiras no estudo de genes envolvidos na resposta à radiação ultravioleta (BAN *et al.*, 2007), a infecções fúngicas e bacterianas (KÜRKCÜOGLU *et al.*, 2007; NORELLI *et al.*, 2009; PARIS *et al.*, 2009) e à abscisão de frutos (ZHOU *et al.*, 2008). Além disto, a geração de bibliotecas subtrativas também foi empregada para o estudo de dormência de gemas com resultados satisfatórios em pessegueiro (LEIDA *et al.*, 2010; JIMÉNEZ *et al.*, 2010), damasco (YAMANE *et al.*, 2008) e chá-verde (KRISHNARAJ *et al.*, 2011).

Tendo em vista a limitada informação gênica sobre a regulação e a sinalização da dormência de gemas (HORVATH *et al.*, 2003; HORVATH, 2009; CAMPOY *et al.*, 2011); aliado aos resultados positivos envolvendo a utilização da técnica de SSH em macieira e no estudo da dormência; e levando-se em conta a possibilidade de exploração do modelo 'Gala' *vs* 'Castel Gala', a técnica de SSH foi a escolhida para a realização da análise da expressão gênica diferencial entre duas cultivares de macieira com requerimento de frio hibernal contrastante (FALAVIGNA, 2010).

Neste estudo, foram construídas quatro bibliotecas supressivas subtrativas recíprocas na entrada (maio) e na saída (agosto) da dormência de ambas as cultivares, as quais tiveram seus transcritos sequenciados e anotados funcionalmente segundo os termos de ontologia gênica (GO, do inglês, *Gene Ontology*). As gemas de 'Gala' mostraram maior número de transcritos relacionados à resposta a estresses bióticos e abióticos. As gemas de 'Gala' de agosto revelaram a presença de transcritos de genes codificadores de fatores de transcrição associados à dormência tais como aqueles pertencentes à família GRAS e DAM, além de proteínas osmoprotetoras como DHN. As gemas de 'Castel Gala' apresentaram enriquecimento de transcritos associados à fotossíntese e ao citoesqueleto (FALAVIGNA, 2010).

Na presente Dissertação está relatada a continuidade dessas atividades visando validar e caracterizar o perfil transcricional de uma coleção de genes candidatos previamente identificados nas quatro bibliotecas subtrativas. Esses genes foram selecionados a partir de análises por bioinformática e de revisão da literatura atual, estando associados ao estabelecimento, à manutenção e à superação da dormência de gemas em macieira. A avaliação da expressão gênica foi realizada pela técnica de reação em cadeia da DNA

polimerase quantitativa precedida de transcrição reversa (RT-qPCR, do inglês, *reverse transcription-quantitative polymerase chain reaction*).

2 OBJETIVOS

2.1 OBJETIVO GERAL

Estudar o requerimento hibernal contrastante entre a cultivar de macieira Gala e sua mutante Castel Gala por meio da exploração da sua expressão gênica diferencial, utilizando a técnica de construção de bibliotecas supressivas subtrativas para a identificação de genes potencialmente associados ao processo de entrada e de saída da dormência de gemas em macieira.

2.2 OBJETIVOS ESPECÍFICOS

- Realizar a validação da expressão de uma coleção de genes candidatos previamente identificados nas bibliotecas supressivas subtrativas, os quais estão potencialmente associados ao estabelecimento, à manutenção e à superação da dormência de gemas em macieira;

- Caracterizar o perfil transcricional dos genes validados em três ciclos anuais de amostragens de gemas dormentes de macieira;

- Identificar genes que possam ser utilizados como marcadores de características específicas do processo de dormência para permitir o acompanhamento da evolução deste processo, bem como a possibilidade de seu uso biotecnológico.

3 CAPÍTULO I

DIFFERENTIAL TRANSCRIPTIONAL PROFILES OF DORMANCY-RELATED GENES IN APPLEBUDS

Manuscrito a ser submetido ao periódico 'Plant and Cell Physiology'

Running title:

Dormancy-related gene expression in apple buds

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Subject area: (3) regulation of gene expression

Number of color figures: 4

Number of tables: 3

Title

Differential Transcriptional Profiles of Dormancy-Related Genes in Apple Buds

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Abbreviations: AP2, APETALA2; ARC5, accumulation and replication of chloroplast 5; ARP6, actin-related protein 6; CAMTA1, calmodulin-binding transcription activator 1; CBF, C-repeat binding factor; CO, CONSTANS; COR, cold-regulated; CR, chilling requirement; CRT, C-repeat; DAM, dormancy-associated MADS-box; DHN, dehydrin; DRE, dehydration-responsive element; DREB, dehydration-responsive element binding protein; EST, expressed sequence tag; FT, FLOWERING LOCUS T; GAST1, GA stimulated transcript 1; GO, gene ontology; GoLS, galactinol synthase; GRAS, GA insensitive, repressor of GA1, scarecrow; ICE1, inducer of CBF expression 1; LEA, late embryogenesis abundant; LOS1, low expression of osmotically responsive genes 1; LTI, low-temperature inducible; LTI65, low-temperature-induced 65; MDH, malate dehydrogenase; NAC, no apical meristem, ATAF1/2, cup-shaped cotyledon 2; RAP2.12, related to APETALA2-12; RCI, rare cold inducible gene; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; SCL, scarecrow-like; SSH, suppression subtractive hybridization; TB, terrific broth; WD40, transcription factor WD40-like repeat domain.

Footnotes:

The nucleotide sequences reported in this paper has been submitted to GenBank under the accession numbers XXX.

Abstract

Considering the limited information about bud dormancy control in apple (*Malus* x *domestica* Borkh.), this work aimed to investigate the differential gene expression profiles between 'Gala Standard' and its derived bud sport 'Castel Gala'. 'Castel Gala' bears a spontaneous mutation which reduced in 50% the chilling requirement for dormancy completion and consequently anticipated budbreak in the field. We performed a suppression subtractive hybridization (SSH) assay, which yielded 28 candidate genes putatively associated with dormancy cycling. RT-qPCR analyses were performed in order to validate the differential expression profiles and also to transcriptionally characterize those genes in three distinct apple cultivars during a cycle comprising growth to dormancy. Among the 28 candidate genes, 17 had the differential expression predicted by SSH confirmed. For several genes, seasonal transcript accumulation during the winter was identified, with the higher steady-state levels of transcript maintained for more time in high chilling requirement cultivars. This profile suggests that those genes may be acting on dormancy regulation. Out of these 17 gene candidates, transcripts coding for dormancy-associated MADS-box (DAM), dehydrins, GAST1, LTI65, NAC, histones H2A.Z and RAP2.12 displayed major differences in gene expression between cultivars through the winter and are strong candidates to play key roles on the dormancy process in apple trees.

Keywords: apple, dormancy, gene expression, *Malus* x *domestica*, RT-qPCR, suppression subtractive hybridization

Introduction

Perennial plants from temperate climate regions go through a dormancy process during autumn and winter which is responsible for the cessation of visible growth. Dormancy is triggered by adverse environmental conditions such as exposure to low temperatures and photoperiodic modifications (Horvath et al. 2003, Rohde and Bhalerao 2007). Bud dormancy in many temperate crops, such as apple (*Malus* x *domestica* Borkh.), is genetically controlled and overcoming this process requires exposure to low temperatures.

Agricultural production of temperate fruit crops in mild-winter climates often face difficulties in obtaining high yields. Success in such production systems demands the use of chemicals or other means to break bud dormancy in order to compensate for insufficient chilling. Modeling of future global climate conditions predicts the rising of global mean temperatures and milder winter temperatures, which could compromise temperate crop yields along the next decades (Campoy et al. 2011). Development of cultivars with low chilling requirements (CR) is a promising alternative to sustain crop production in a changing environment. The understanding of the mechanisms responsible for this phenotype may permit the development of new strategies to reach this goal.

Dormancy release and cold perception, the last being central in dormancy progression, are still poorly understood processes at the molecular level in plants. Dormancy entrance, on the other hand, especially when triggered by short days, is quite well characterized. In *Arabidopsis* CONSTANS (CO) and FLOWERING LOCUS T (FT) are proteins responsible for daylenght perception and flowering, respectively, but in aspen trees the *CO/FT* module also regulates dormancy establishment (Böhlenius et al. 2006). In poplar, dormancy entrance involves a deep transcriptional and metabolic reprogramming, directing cellular resources towards synthesis of osmoprotectors and cold acclimation-related proteins, all orchestrated mainly by ABA and ethylene (Ruttink et al. 2007). In peach, a family of dormancy-associated MADS-box genes (DAM) is required for growth cessation and dormancy establishment (Bielenberg et al. 2008). A recent review proposes a molecular model for dormancy control, where DAM proteins are capable to repress *FT* expression (Horvath 2009). In this model, day length regulates several genes through the action of circadian clock and the *CO/FT* module (Böhlenius et al. 2006, Horvath 2009). The CBF pathway, which in *Arabidopsis* is involved in response to cold stresses, may also participate on dormancy regulation inducing *DAM*

genes (Horvath 2009, Kurbidaeva and Novokreshchenova 2011). Chromatin remodeling process helps the regulation of these genes during bud dormancy (Horvath 2009).

Apple tree is another example of perennial tree that presents bud dormancy during winter months. However, different apple cultivars may have distinct CRs to overcome this process (Heide and Prestrud 2005, Jackson 2003). This seems to be the case of the Castel Gala cultivar, which was originated from a spontaneous bud sport mutation of a 'Gala Standard' apple tree. 'Castel Gala' features low CR – 400 hours – when compared to the original cultivar – 800 hours (Anzanello et al. 2010, Denardi and Seccon 2005). This new cultivar exhibits approximately 25 days shorter cycle of budburst, flowering and fruit harvesting, producing fruits anatomically and nutritionally very similar to 'Gala Standard' (Denardi and Seccon 2005). Therefore, genes associated with lower CR may be useful for the development of cultivars with better adaptation to environmental conditions which could lead to higher fruit production.

Apple bud dormancy regulation differs from better studied dormancy models, such as poplar and peach, in the sense that it is not triggered by short photoperiods (Heide and Prestrud 2005). Dormancy in apples is well-characterized physiologically (Faust et al. 1997), and a major QTL locus for flowering time has been found (Van Dyk et al. 2010, Celton et al. 2011). However, no transcriptional information about dormancy control in apple buds is available. The public release of the apple genome (Velasco et al. 2010) and the available apple EST databases (over 300,000 sequences) provided good frameworks for apple genomics. Considering the available resources and the model 'Gala Standard' versus 'Castel Gala', we sought to obtain and characterize transcriptional information on apple dormancy regulation.

In this work, the suppression subtractive hybridization (SSH) technique was used to perform a differential gene expression study in apple buds of Gala Standard and Castel Gala cultivars aiming to identify genes involved in dormancy establishment, maintenance and release. Four SSH libraries were constructed and the expression patterns of 28 selected genes were analyzed by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) for validation.

Results

SSH library construction

Aiming to explore the molecular potential of dormancy progression between the CR contrasting apple tree cultivars ‘Gala Standard’ and ‘Castel Gala’, dormant buds of ‘Gala Standard’ (G) and ‘Castel Gala’ (K) harvested in May (1) and August (2) at the city of Monte Castelo (SC) were used to construct four SSH libraries representing genes in dormancy entrance (G1-K1 and K1-G1), dormancy maintenance (G2-K2) and dormancy release (K2-G2). Tester and driver cDNAs were used as shown in Table 1. The SSH technique yielded 4,241 clones of putative differentially expressed genes, with fragment sizes ranging from ~100 to 700 bp. 384 clones from each library (1,536 total) were sequenced, and the high-quality sequences obtained (1,359) were assembled into 1,019 unigenes. All ESTs and unigenes were functionally annotated by BlastX at the NCBI and Gene Ontology (GO) terms. Annotated ESTs were submitted to GenBank. Best BlastX hits and significant GO terms of assembled sequences and singlets are presented in Supplementary Table S1. The most frequent GO terms belonging to biological process domain of each library are depicted in Fig. 1. Among the most frequent terms identified, some were unique for each library as ‘response to endogenous stimulus’ and ‘reproduction’ for K1-G1, ‘signal transduction’ for G1-K1, ‘photosynthesis’ for K2-G2, and ‘biosynthetic process’ for G2-K2 (Fig. 1 and Supplementary Table S1).

Fisher’s exact tests of EST functional annotations revealed significantly enriched terms for each library (Table 2). At the sampling point of May 2007 (G1-K1 and K1-G1) both cultivars were fully dormant. Only two GO terms were found enriched in this ‘Gala Standard’ samples, ‘small molecule metabolic process’ and ‘cellular amino acid and derivative metabolic process’, which reflected the amount of metabolism-related terms (34) significantly enriched when more specific terms were tested (data not shown). The SSH K1-G1 subtraction showed less enrichment of specific terms compared to G1-K1, with only 12 terms metabolism-related (data not shown). No terms were found enriched by the “slim” method in these ‘Castel Gala’ samples. In the sampling point of August 2007 (G2-K2 and K2-G2), when ‘Gala Standard’ buds were still dormant and ‘Castle Gala’ buds were already developing, there was a clear functional distinction between both EST sets. ‘Gala Standard’ samples were significantly enriched in transcripts related to response to abiotic stress and

carbohydrate metabolism, while 'Castel Gala' ESTs were enriched in terms related to cell parts and photosynthesis (Table 2).

Validation of apple CR-related candidate genes by RT-qPCR

From the 1,019 unigenes identified by SSH, we selected 28 as candidates based on bioinformatics analysis and the current knowledge of dormancy regulation (Supplementary Table S2). Among the 28 selected apple unigenes are those exhibiting high homology to known genes involved in cold signaling and acclimation in *Arabidopsis*, like those coding for calmodulin-binding transcription activator (CAMTA1), inducer of CBF expression 1 (ICE1), no apical meristem/ATAF2/cup-shaped cotyledon 2 (NAC), ESKIMO1, low expression of osmotically responsive genes 1 (LOS1), low-temperature induced (LTI), LTI65, rare cold-induced (RCI), and dehydrins (DHNs; Kurbidaeva and Novokreshchenova 2011), as well as galactinol synthase (GoLS; Taji et al. 2002) and trehalose-6-phosphate synthase (T6PS; Penna 2003). Genes encoding scarecrow-like (SCL), GA-stimulated transcript 1 (GAST1), DELLA and other GA insensitive/repressor of GA1/scarecrow (GRAS) proteins were also identified, and these are known to participate in gibberellin signaling and regulation (Shi et al. 1992, Lee et al. 2008). Also present were putative transcripts coding for actin-related protein 6 (ARP6) and histones, that are described to participate in chromatin remodeling and cell growth. More recently, the H2A.Z histone variant was described as being temperature-sensitive (Kumar and Wigge 2010). Genes for *APETALA2* (AP2) and *CO* participate in floral organ development (Böhlenius et al. 2008, Yant et al. 2010). The *FACKEL* gene detected is known to play a crucial role in plant cell division, embryogenesis and development (He et al. 2003). A gene coding for a protein related to AP2.12 (RAP2.12) was among the selected ones and it was recently reported to have a central role in hypoxia signaling (Licausi et al. 2011). An alcohol dehydrogenase (ADH) gene was present and such genes were previously related to dormancy in grape (Or et al. 2000), also playing roles during hypoxia in *Arabidopsis* (Bailey-Serres et al. 2012). Finally, putative *DAM* genes were among the SSH selected candidates. These genes are known to be regulatory players in the dormancy establishment and release in peach (Bielenberg et al. 2008, Jiménez et al. 2010a).

Since the SSH procedure may yield false positives (Kuhn 2001), we reassessed their expressions by RT-qPCR employing the same original RNA samples (2007) and a second group of RNAs extracted from terminal buds harvested in 2008 from 'Gala Standard' and

‘Castel Gala’ apple trees located in the same experimental field in Monte Castelo (SC). The differential gene expression between cultivars was considered validated when the pattern observed in the SSH was confirmed in the samples of both years by RT-qPCR with statistical significance ($p<0.05$).

The differential expression was confirmed for 17 out of the 28 tested genes, namely *ADH*, *GAST1*, the three *DHN*s, two histones H2A variant H2A.Z, *LTI65* and the transcription factors *AP2*, *DAM*, *GRAS*, *ICE1*, *NAC*, *RAP2*.12 and *SCL* (Fig. 2). Most interestingly, transcripts for two genes were found and validated in subtractions from both sample points. Expression of a *LTI65* gene was higher in ‘Gala Standard’ at both harvesting points, and the gene encoding a putative histone H2A.Z variant, *HTA8*, was more expressed in G1-K1 and K2-G2 libraries (Fig. 2). Contrarily, eleven SSH-selected candidate genes were not validated by RT-qPCR, namely *ARP6*, *CO*-like, *DELLA*, *ESKIMO*, *FACKEL*, *HTA11*, *LOS1*, *LTI*, *RCI1*, *RCI2* and *T6PS* (Supplementary Fig. S1). All validated genes had their transcript accumulation profiles analyzed by RT-qPCR in dormant bud samples of three different apple tree cultivars over 2009/2010.

Characterization of CR-related candidate genes by RT-qPCR in different apple cultivars

To better characterize the transcript profiles of the selected17 differentially expressed genes along the dormancy cycle in additional apple cultivars, we used a color mutation of ‘Gala Standard’, namely ‘Royal Gala’. This cultivar is largely cultivated around the world, since it produces fruits less susceptible to bruising and more uniformly colored. Therefore, ‘Gala Standard’ and ‘Royal Gala’ show similar patterns of bud dormancy, with quite equivalent CRs (Walsh and Volz 1990, EPAGRI 2006).

We performed RT-qPCRs with RNA samples extracted from closed buds harvested over 2009 and 2010 of ‘Royal Gala’ and ‘Castel Gala’ trees in a second location (Papanduva, SC). The amount of 450 chilling hours accumulated by ‘Royal Gala’ and ‘Castel Gala’ samples in July 2009 (Table 3) was known to be sufficient to break the dormancy of ‘Castel Gala’ buds but not ‘Royal Gala’ buds, as determined in experimental conditions (Anzanello et al. 2010). Accordingly, ‘Castel Gala’ buds were visibly more advanced towards dormancy completion in that date as observed by the presence of a higher number of silver tips (which were not sampled).

The quantitative expression analysis of the 17 previously validated genes by RT-qPCR on samples harvested in 2009/2010 revealed that ten genes exhibited the same pattern of transcript accumulation observed by SSH in ‘Royal Gala’ and ‘Castel Gala’ samples, namely *GAST1*, the three *DHN*s, two histones H2A variant H2A.Z, *LTI65* and the transcription factors *DAM*, *NAC* and *RAP2.12* (Fig. 3). *GAST1* and *Mddhn* genes showed the most contrasting levels of steady-state mRNA between ‘Royal Gala’ and ‘Castel Gala’ in samples harvested in July 2009 (winter), with much higher levels in ‘Royal Gala’ than in ‘Castel Gala’, and dropping to similar levels in the beginning of summer (November/December 2009; Fig. 3). In accordance, transcripts for these two genes were among the most redundantly sequenced in the pool resulting from the G2-K2 subtraction (seven and 25 times, respectively; data not shown). Although not as prominent, genes putatively coding for DAM, MdDHN1, DHN-like and NAC proteins also exhibited higher transcript levels for ‘Royal Gala’ in July 2009 than ‘Castel Gala’, lowering in summer samples. Contrarily, genes putatively encoding both histones and RAP2.12 proteins exhibited higher transcript levels in ‘Castel Gala’ samples harvested in July than ‘Royal Gala’, reversing levels in the months of summer (Fig. 3). Differing from all others, the *LTI65* gene exhibited differences in transcript levels only in samples harvested in the end of autumn 2009 (May), being higher in ‘Castel Gala’ than ‘Royal Gala’. Similar levels of transcripts for this gene were observed in both cultivars from June 2009 to April 2010 (Fig. 3).

The transcript profiles of the other seven validated genes, namely *ADH*, *AP2*, *CAMTA*, *ICE1*, *GoLS*, *GRAS* and *SCL*, showed a different pattern from the one exhibited by SSH in ‘Royal Gala’ and ‘Castel Gala’ samples over 2009/2010 by RT-qPCR. The genes enconding for ADH, AP2, CAMTA1, ICE1 and GRAS displayed a peak of transcript accumulation for ‘Castel Gala’ in July samples (Supplementary Fig. S2). The expected profile would be ‘Royal Gala’ displaying more accumulation in this sample point. Similar levels of transcripts for these genes were observed in both cultivars in other sampling points. The genes enconding for GoLS and SCL had similar transcript accumulation for both cultivars over 2009/2010 sampling points (Supplementary Fig. S2).

As the experimental area in Papanduva (SC) belongs to a commercial orchard, dormancy release was forced with the application of hydrogen cyanamide, which was done after our sampling. This explains the lack of sampling dates between July and November 2009. To overcome this large gap between sampling times, closed buds of ‘Fuji Standard’ trees from a third experimental area (in Caçador, SC) were also harvested. This is a high CR

cultivar (Botelho et al. 2006) and the transcriptional profile of the selected genes in these samples could confirm patterns associated with a low or high CR. No hydrogen cyanamide was applied in Caçador orchard. The same 17 genes whose transcript levels allowed their identification by SSH between 'Gala Standard' and 'Castel Gala', being confirmed by RT-qPCR, were assayed by RT-qPCR in closed bud samples harvested from apple 'Fuji Standard' trees. Most genes showed very similar seasonal patterns of transcript accumulation observed in the 'Royal Gala' samples previously described (Fig. 4 and Supplementary Fig. S3). Interestingly, the transcriptional profiles for the ten genes described above corresponded very closely in the comparison between Royal Gala and Fuji Standard cultivars (Figs. 3 and 4), while four out of the seven remaining genes were expressed in a very similar fashion between 'Fuji Standard' and 'Castel Gala' (Supplementary Figs. S2 and S3).

Discussion

'Gala Standard' *vs* 'Castel Gala' model

Naturally occurring genetic variation provides a good starting point for characterizing biological phenomena, and differential expression screening may point to the molecular mechanisms associated to such variance. Besides the research opportunity, crop genetic variation is the basis for the establishment of new varieties (Arora et al. 2003, Fernie et al. 2006).

The apple tree 'Castel Gala' was discovered and characterized as an early flowering cultivar directly derived from a 'Gala Standard' background. 'Castel Gala' shows approximately half of the CR need by 'Gala Standard' or 'Royal Gala' for bud dormancy release, and it allowed improving adaptation to subtropical climate (Denardi and Seccon 2005, Anzanello et al. 2010). 'Castel Gala' budburst is anticipated over a month in relation to 'Gala Standard' (or 'Royal Gala') at field conditions. With this model of contrasting bud dormancy in apple trees at hand, we aimed to find clues to the molecular regulation of such alteration. We chose the SSH technique since it has being used successfully in apple to characterize a wide range of biological processes, including responses to ultraviolet radiation (Ban et al. 2007), bacterial and fungal infection (Degenhardt et al. 2005, Kürkcüoglu et al. 2007, Norelli et al. 2009, Paris et al.2009) and regulation of fruit abscission (Zhou et al. 2008). SSH of cDNA libraries has also been recently used for bud dormancy studies with

satisfactory results in peach (Leida et al. 2010, Jiménez et al. 2010b), Japanese apricot (Yamane et al. 2008) and tea (Krishnaraj et al. 2011).

Functional enrichment

Despite the distinct dormancy levels displayed by the buds used in the construction of the four SSH libraries, their sequencing and functional characterization showed interesting connections between them. Interestingly, closed buds from K2-G2, which already started dormancy release, exhibited a pool of cDNAs characterized by the same GO terms of those cDNAs from fully dormant libraries, as G2-K2 (Fig. 1). Except for some exclusive GO terms attributed to some groups of cDNAs for each cultivar, their transcripts basically shared the same GO terms attributes. Other works exploring the SSH technique to study dormancy, already found subtractions with transcripts belonging to a broad range of functions (Yamane et al. 2008, Leida et al. 2010, Jiménez et al. 2010b, Krishnaraj et al. 2011). Therefore, this equivalence in functions found here may indicate that basal metabolic pathways are equally active throughout the dormancy cycle.

The Fisher's exact tests allowed us to compare our ESTs sets to an external dormant bud EST library (LIBEST_015808 (AAMA) Royal Gala spur bud autumn, GenBank; Table 2). This tool is useful to identify GO terms over-represented between two EST sets (Conesa et al. 2005). The four pools of subtracted transcripts showed profiles consistent with the expected physiological stage of the buds. The G1-K1 subtraction was generally enriched in metabolism-related GO terms. This result is analogous to the one found in poplar, where massive metabolic changes take place during dormancy entrance, at the same time that bud tissues cold-acclimate (Ruttink et al. 2006). The SSH transcript pool resulting from the G2-K2 subtraction also presented GO term enrichment towards metabolism, but in addition, GO terms related to stress response were overrepresented in still dormant buds derived from 'Gala Standard' harvested in August 2007. This could be a result of the high redundancy of *DHN* transcripts, which accounted for more than 10% of the transcripts obtained after the G2-K2 subtraction (data not shown). The identification of GO terms related to stress response in this subtraction fits well with the physiological stage of the buds used in this construction, 'Gala Standard' was in the middle of the dormancy process, as also with results found using SSH to study dormant buds in peach (Leida et al. 2010). The K2-G2 subtraction showed a significant number of GO terms related to growth and photosynthesis. 'Castel Gala' buds

flushed six days after the harvesting of these samples, hence the transcriptional program at the harvesting date reflected the preparation for growth resumption (Table 2).

Validation of apple CR-related candidate genes by RT-qPCR

Although the SSH technique is largely being used over the years in the study of differentially expressed genes, this procedure may yield false positives (Kuhn 2001). Our objective was to identify many genes as possible exploiting the 'Gala Standard' *vs* 'Castel Gala' model. With this purpose, we sequenced a wide range of clones obtained by the SSH assay and functionally characterized these EST with bioinformatics analyses. With the aim to confirming the differential expression of selected genes, we evaluated their expressions by RT-qPCR using RNA samples of closed buds harvested in 2007 and 2008 from 'Gala Standard' and 'Castel Gala' apple trees located in the same experimental field used to harvest plant material for the SSH in Monte Castelo (SC). From the 28 selected genes, only 17 displayed the same expression pattern previously identified, but this amount of validated genes was higher if compared to other similar studies. A recent study using SSH to address a list of dormancy-related genes in peach, found out that only a low percentage of the clones obtained were validated, around 15 to 48% (Leida et al. 2010). Similar results were obtained studing bud dormancy in Japanese apricot (Yamane et al. 2008). These 17 validated genes were profiled by RT-qPCR in dormant bud samples over 2009/2010 of three different apple tree cultivars displaying distinct CRs.

Characterization of CR-related candidate genes by RT-qPCR over 2009/2010

With the aim to confirming the differential expression of validated genes, we performed the transcriptional profiling of closed buds harvested in various times of the 2009/2010 cycle. It would be expected for a dormancy-related gene to be expressed mainly at late autumn and winter, and, in fact, this was observed for most of the validated genes. In addition, the comparison of transcripts of buds samples from Royal Gala and Castel Gala apple cultivars growing in the same field conditions in Papanduva (SC) was a good opportunity to further study the differential expression found by the SSH technique, especially for July/2009 samples. These buds were harvested in a time point when 'Castel Gala' trees are generally in a more advanced stage of dormancy, although not in budburst.

Also, as the ‘Fuji Standard’ sampling was more complete during the 2009/2010 cycle, the dormant buds harvested in Caçador (SC) were useful to confirm gene profiles associated with a low or high CR.

Among the 17 candidate genes, the analysis of transcript accumulation over 2009/2010 samples in three distinct cultivars may permit the identification of gene profiles that could be addressed to roles in dormancy regulation. Although the genes profiled here belong to a wide range of cellular functions, such as cold-responsive, gibberellin signaling, dormancy-associated, hypoxia signaling and chromatin remodeling, we could identify several transcripts sharing the same profiles. This may suggest that many pathways are interacting during dormancy progression.

In the model plant *Arabidopsis thaliana* a set of cold-regulated genes is responsive to the CBF/DREB1 transcription factors (Thomashow 2010). The concerted action of these proteins regulates the expression of cold-related (COR) genes, which confers cold-tolerance (Kurbidaeva and Novokreshchenova 2011). Most of these *COR* genes are included in the LEA family, characterized by conferring tolerance to drought and low temperatures (Hundertmark and Hincha 2008). In this work, several genes that participate in the *CBF/DREB1* pathway were identified and validated, such as *CAMTA1*, *DHN*s, *GoLS* and *ICE1*.

A. thaliana DHNs, members of the LEA family, are well-characterized proteins and in apple play important roles in bud dormancy (Faust et al 1997). We identified three *DHN* genes that displayed interesting profiles over 2009/2010. Out of the three *DHN* genes identified in our study, the most highly expressed in dormant buds was described elsewhere as midwinter expressed (*Mddhn*; Garcia-Bañuelos et al 2009). Another transcript identified in our study was already identified in an EST collection as a cold induced DHN and labeled *Mddhn1* (Wisniewski et al. 2008). The remaining gene seems to encode a novel apple *DHN* gene, namely *Mddhn*-like. Promoter analysis from genomic data (Velasco et al. 2010) revealed three conserved DRE/CRT motifs in the promoter region of *Mddhn*, two in the promoter of *Mddhn*-like and one in the promoter of *Mddhn1* (data not shown). Interestingly, the amount DRE/CRT motifs followed the same trend of the transcriptional induction level found during winter (Fig. 3 and 4).

The apple *Mddhn* gene displayed an interesting transcriptional pattern among the three cultivars analyzed during 2009/2010. ‘Castel Gala’, the low CR cultivar, had decreased *Mddhn* transcript level first, near its budbreak date. The other two high CR cultivars, which

stayed dormant until September, kept the high transcription level until spring (Faust et al. 1997). Apple buds, in particular, reduce their free water content during dormancy and DHN proteins were described to accumulate during winter (Faust et al. 1997). DHNs have roles in membrane stability and therefore may help stabilizing cellular structures, especially during osmotic stress, which is well known to happen in dormant buds (Faust et al. 1997, Erez et al. 1998, Kosová et al. 2007). The seasonal profile observed in all three cultivars (Figs. 3 and 4) suggests that these genes may be playing important roles in osmotic stress. Also, *Mddhn* could be used as a molecular marker of dormancy progression in apple trees (Garcia-Bañuelos et al 2009).

Besides gene encoding DHNs, CBF/DREB1 transcription factors induce transcription of genes encoding enzymes related to osmolytes and compatible solutes production (Zhu et al. 2007). Accumulation of these compounds is a well-known response to cold, freezing and water deficit. Some osmoprotectants in plants belong to the raffinic series (Klotke et al. 2004). The biosynthesis of raffinic sugars begins with the production of the building block galactinol by galactinol synthase. In our study, for the first time in dormant apple buds, a galactinol synthase transcript (GoLS) is found highly enriched. In *Arabidopsis*, one galactinol synthase gene (*AtGolS3*) is induced by cold treatment and *CBF/DREB* overexpression but not by drought or excess salt (Taji et al. 2002, Maruyama et al. 2009). The transcriptional profiles obtained for this gene in all three cultivars suggests a midwinter expression closely related to dormancy (Supplementary Figs. S2 and S3; Unda et al. 2012). The comparison of galactinol synthase transcription and raffinose sugar levels during dormancy cycling would help clarify possible roles of this pathway.

In addition to genes downstream of the *CBF/DREB1* pathway, we identified two transcripts putatively upstream of it, represented in the G2-K2 subtraction, namely as *ICE1* and *CAMTA1*. Both genes encode *trans*-acting proteins capable of transducing cold signals (Kurbidaeva and Novokreshchenova 2011). Differential expression of genes similar to *ICE1* during dormancy was reported for at least two other plants (Leida et al. 2010, Horvath et al. 2008). This seems to be the first time that *CAMTA1* was identified in a dormancy-related study. However, for these two genes, no clear relation between the transcript accumulation in both high CR cultivars was observed (Supplementary Figs. S2 and S3). Other levels of regulation may account for gene expression modulation, and at least for ICE1 post-translational modifications are known to be triggered by cold exposure (Miura et al. 2007).

The overall data suggests participation of the *CBF/DREB* regulon during the maintenance of apple bud dormant state (Wisniewski et al. 2011).

Gibberellin signaling is long agreed as important to dormancy progression. Ectopic application of active gibberellins can substitute chilling exposure (Hartmann et al. 2011, van der Schoot and Rinne 2011). Recently, a study provided a mechanistic view of dormancy release as reestablishment of symplastic connections by gibberellin-induced degradation of plasmodesmal callose (Rinne et al. 2011). In our work, at least three genes potentially related to gibberellin signaling, *GAST1*, *GRAS* and *SCL*, were found enriched in dormant buds. *GAST1* homolog showed a striking contrast of expression level between 'Royal Gala' and 'Castel Gala' buds in May and July 2009 (Fig. 3). Although the exact function of GAST1 is unknown, its reported responsiveness to gibberellins suggests involvement in the signaling of this hormone (Shi et al. 1992).

The GRAS protein family and its members SCL are transcription factors involved in global functions, like growth, development, GA signaling and light signal transduction (Lee et al. 2008). In our study, transcripts coding for GRAS and SCL proteins were identified and validated in 2007 and 2008 as enriched in G2-K2 library (Fig. 2). This pattern was not observed in 2009 samples (Supplementary Fig. S2). However, real time analysis detected significantly higher expression of both genes in 'Gala Standard' and 'Royal Gala' May samples across the three years (Fig. 2 and Supplementary Fig. S2). These data, together with *GAST1* transcript profiles, suggest that the genetic event that conferred 'Castel Gala' a low-CR may have affected gibberellin metabolism and/or signaling.

DAM genes were first described in the study of the peach cultivar 'Evergrowing', which does not enter into dormancy even under arrest-inducing conditions (Bielenberg et al. 2008). This cultivar has a deletion spanning six StMADS11-clade MADS box genes, considered responsible for the trait. *PpDAM5* and *PpDAM6* have expression patterns consistent with a growth-repressing role, increasing at the onset of arrest and declining through the winter (Li et al. 2009). Similar genes were found in pear (Ubi et al. 2010), Japanese apricot (Sasaki et al. 2011) and kiwifruit (Wu et al. 2012), but to date no *DAM* gene has been described for apple.

'Gala Standard' buds revealed a *DAM*-like gene with high level of expression during the dormancy progression (Fig. 2). The transcript profiles for this gene over 2009/2010 displayed a dormancy-related expression in all three cultivars analyzed, as it strongly decreased in the summer in a very similar fashion to peach *DAM5* and *DAM6* genes (Figs. 3

and 4; Li et al. 2009). Also, the low CR cultivar Castel Gala had the transcript accumulation downregulated first in comparison to high CR cultivars Royal Gala and Fuji Standard. In an independent study, our group identified six putative *DAM* genes in *in silico* searches of *Malus* x *domestica* predicted transcripts (Velasco et al. 2010). Four of them showed seasonal and cold regulated transcription, resembling peach *DAM6*, and the *DAM* gene found in this study is one of them (Porto et al. unpublished data). Peach and apple are evolutionarily close (Illa et al. 2011) hence it is possible that the *DAM* gene described here participates in dormancy establishment in apple analogously to *DAM* genes in peach.

Besides a *DAM* gene, at least two other potential transcription factors were found enriched in G2-K2 library, namely a NAC and an AP2 domain-containing protein. Transcripts coding for similar transcription factors were found in dormant buds of peach by SSH (Leida et al. 2010). The NAC gene showed a profile similar to the *DAM* gene in 2009/2010 samples, with a fall of transcript accumulation prior to budbreak both in 'Castel Gala' and in 'Fuji Standard' samples (Figs. 3 and 4), suggesting a role in dormancy maintenance.

Hypoxic conditions involve alternative ways of respiration (Bailey-Serres et al. 2012). Dormancy breaking of grape buds induces the expression of a range of hypoxia-related genes such as alcohol dehydrogenase (ADH) and pyruvate decarboxylase (PDC; Ophir et al. 2009, Or et al. 2000). Recently, a key transcription factor in hypoxia signaling, RAP2.12, was described in *A. thaliana* (Licausi et al. 2011). RAP2.12 protein directly senses low oxygen concentrations and induces the expression of hypoxia-related genes. An unigene very similar (62.2%) to RAP2.12 was found in the G1-K1 subtraction and is recurrently more expressed in 'Gala Standard' and 'Royal Gala' than in 'Castel Gala' during autumn (Fig. 2 and 3). This profile could be associated to the dormancy release process (Ophir et al. 2009, Or et al. 2000). Also, we identified a transcript coding for ADH which displayed a peak of expression near the budbreak of 'Castel Gala' buds (Supplementary Fig. S2). Analyzing the profiles observed for 'Royal Gala' and 'Fuji Standard' samples it's noticeable a linear expression along the year of 2009. This pattern could be explained by the transient nature of expression of this gene. In grape, *ADH* expression decreased after four days from the start of the respiratory disturbance (Or et al. 2000). Since our sampling interval is one or several months, it may not have detected this discrete induction.

Histones in general are central players in cell division and DNA metabolism where some histone variants may have additional roles, as transcriptional control. H2A.Z variants of

A. thaliana are destabilized during temperature shifts from 17 to 27°C and exposing DNA to regulatory factors (Kumar and Wigge 2010). The K2-G2 subtraction had a lot of ESTs and unigenes matching to histones, and because of the potential relevance to both cell division and temperature perception we decided to further investigate histone transcripts matching *A. thaliana* H2A.Z variants. Interestingly, a gene product that may be part of H2A.Z deposition in chromatin, *ARP6*, was found in the opposite subtraction, G2-K2, however was not validated by RT-qPCR. Both histone transcripts identified showed higher expression in 'Castel Gala' buds close to budburst, in agreement with a function in cell division and growth (Fig. 3). Participation of these apple histones in temperature-driven DNA metabolism remains to be investigated.

Finally, we also validated and analyzed transcript accumulation for the *LTI65* gene, which still does not have a known function described. Besides that, the transcriptional profile observed for this gene over 2009/2010 is very interesting (Figs. 3 and 4). The seasonal transcription profiles for this gene in the three cultivars analyzed were very similar, suggesting an environmental control of expression. Also, this gene showed the highest levels of expression during the winter compared to summer.

Natural genetic variants can be reliable models for the study of complex traits, and SSH library construction is a cost-efficient and powerful technique to explore these models. Trying to unveil the genetic event that resulted in the low CR cultivar 'Castel Gala' we identified several cue pathways that may be responsible for this trait. The transcriptional profiles obtained for several genes during the 2009/2010 cycle suggest that downregulation of genes involved in dormancy maintenance, as also upregulation of genes involved in dormancy release are being anticipated in 'Castel Gala'. Yet, the differential regulation of transcripts related to gibberellin signaling suggests that this pathway strongly influences the CR in apple trees.

In conclusion, 17 genes belonging to a broad range of functions were identified as differentially expressed in apple buds from contrasting chilling-requirement cultivars. The unigenes annotated as CAMTA1, histones H2A.Z, GoLS, RAP2.12 and LTI65 were identified in this study for the first time as related to dormancy, while the other transcripts had similar counterparts in previously described gene expression studies of dormancy progression in other species (Leida et al. 2010, Horvath et al. 2008, Yamane et al. 2008). Out of these 17 candidates, *DAM*, *DHN*s, *GAST1*, *NAC*, histones *H2A.Z* and *RAP2.12* genes

showed the most consistent differential expression across cultivars and seasons, which confirm their potential as participants in dormancy regulation.

Material and Methods

Plant material

All samples collected consisted of fully closed terminal buds of apple (*Malus* x *domestica* Borkh.) at the phenological stage 'A' according to Fleckinger scale (EPPO, 1984). Terminal buds were harvested from apple trees located in three different experimental areas of the Santa Catarina State (Brazil). Harvested material was immediately frozen and kept in liquid nitrogen until storage at -80°C.

The first experimental area belongs to a commercial orchard in Monte Castelo (26° 37' 39" S, 50° 14' 7.73" W and 791 m altitude) and samples harvested consisted of buds from six 'Castel Gala' and two 'Gala Standard' trees. These six 'Castel Gala' individuals are the ones where this cultivar first appeared. Trees were grafted on Marubakaido rootstock in 2001 (6- or 7-years-old at the harvesting dates). Dormant buds from both cultivars were harvested on May 29th and August 13th 2007. In these both sampling points the Gala Standard cultivar was fully dormant. On the other hand, 'Castel Gala' was already breaking dormancy at the August sampling. We used these contrasting dormancy level samples for SSH library construction (Table 1) as also for RT-qPCR studies. Dormant buds from the same trees were also sampled on May 29th and July 29th 2008 (for RT-qPCR studies) with the same phenological pattern from previously sampled buds. At both years, budburst of 'Castel Gala' happened one week after August harvesting point, while budbreak of 'Gala Standard' occurred nearly 45 days after this sampling.

The second experimental area belongs to a commercial orchard in Papanduva (26° 26' 68" S, 50° 5' 47" W and 788 m altitude). Samples consisted of three biological replicates each containing 20 'Castel Gala' or 'Royal Gala' plants. 'Castel Gala' plants were 3-years-old and grafted on M9 rootstocks. 'Royal Gala' trees were 6-years-old and grafted on Marubakaido rootstocks with M9 filter. Dormant buds from both cultivars were sampled at six different dates from May 2009 until April 2010. Chilling hours accumulated by the 2009/2010 samples are depicted in Table 3. RNAs extracted from all buds harvested were employed in RT-qPCR studies.

The third experimental area belongs to an experimental orchard in Caçador (26º 49' 10" S, 50º 59' 25" W and 935 m altitude) and samples consisted of three biological replicates from four 'Fuji Standard' plants. Trees were 7-years-old and grafted on M7 rootstocks. Harvesting was done at eight different dates from January 2009 until February 2010, including the dormancy progress and the vegetative cycle. Chilling hours accumulated by the 2009/2010 samples are depicted in Table 3. RNAs extracted from all buds harvested were also employed in RT-qPCR studies.

RNA extraction

All RNA extractions were done from 20 frozen buds per sample (approximately 200 mg) by LiCl precipitation using Zeng and Yang (2002) modified protocol with purification scale adapted to 2 ml tubes. After the chloroform extraction step, each extraction was conducted using three 1.5 mL microcentrifuge tubes that had their volumes united before the LiCl precipitation step.

Complementary DNA library construction and SSH

Messenger RNA from the four groups of terminal bud samples harvested in 2007 (Table 1) were isolated from 150 μg total RNA using the Poly(A) Purist Kit (Ambion) according to manufacturer's protocol. Subtractions were performed using PCR-Select cDNA Subtraction Kit (Clontech) according to the manufacturer's protocol, except for the cDNA purification steps. For this purpose, we used QIAquick PCR Purification Kit (Qiagen) according to the manufacturer's instructions. SSH was performed using tester and driver cDNAs as shown in Table 1. Subtracted cDNAs were ligated into pGEM®-T-Easy vector (Promega) and transformed in TOP10F´ Chemically Competent *Escherichia coli* cells (Invitrogen). After growing on plates containing SOC medium, white colonies were picked up and incubated overnight in 96-well plates containing TB-ampicillin and stored at -80°C for further use.

DNA sequencing and EST analysis

Plasmid DNA was extracted by miniprep alkaline lysis protocol and sequenced in the ACTGene Laboratory (Centro de Biotecnologia, UFRGS, Porto Alegre, RS, Brazil) using the automatic sequencer ABI-PRISM 3100 Genetic Analyzer armed with 50 cm capillaries and POP6 polymer (Applied Biosystems). DNA templates (30 to 45 ng) were labeled with 3.2 pmol of the primer 5'-GTAAAACGACGGCCAG-3' and 2 μL of BigDye Terminator v3.1 Cycle Sequencing RR-100 (Applied Biosystems) in a final volume of 10 μL. Labeling reactions were performed in a GeneAmp PCR System 9700 (Applied Biosystems) termocycler with an initial denaturing step of 96 ºC for 3 min followed by 25 cycles of 96 ºC for 10 sec, 55 ºC for 5 sec and 60 ºC for 4 min. Labeled samples were purified by isopropanol precipitation followed by 70% ethanol rinsing. Precipitated products were suspended in 10 μL formamide, denatured at 95 ºC for 5 min, ice-cooled for 5 min and electroinjected in the automatic sequencer. Sequencing data were collected using the software Data Collection v1.0.1 (Applied Biosystems) programmed with the following parameters: Dye Set "Z"; Mobility File "DT3100POP6{BDv3}v1.mob"; BioLIMS Project "3100_Project1"; Run Module 1 "StdSeq50_POP6_50cm_cfv_100"; and Analysis Module 1 "BC-3100SR_Seq_FASTA.saz".

EST visualization and trimming, as well as contig assembly, were performed with CodonCode Aligner (Licor Inc). Sequences shorter than 100 bp, with low PHRED quality (<20), or with the presence of both M13 primer pairs were excluded from the analysis. Sequences were annotated by comparison with the NCBI non-redundant public database with the BlastX algorithm (Altschul et al. 1990) using an estimated (E) value cut-off of 10^{-6}.

Enrichment of Gene Ontology (GO) terms was determined by two-tailed Fisher's Exact tests using a 'Royal Gala' bud EST library as a reference (LIBEST_015808 (AAMA) Royal Gala spur bud autumn, GenBank). Some tests employed the "GO slim" function to identify more general terms. Blast, gene ontology annotation from both ESTs and unigenes and Fisher tests were perfomed using Blast2GO software (Conesa et al. 2005).

RT-qPCR analyses

DNA contamination of total RNA samples was removed using TURBO DNA-free Kit (Ambion) according to manufacturer's protocol. Complementary DNAs were synthesized using GeneAmp RNA PCR Core Kit (Applied Biosystems) according to manufacturer's instructions. Twenty-eight gene-specific primers were designed for each selected candidate

gene using Primer3 v.0.4.0 software (Rozen and Skaletsky 2000) and Oligo Analyzer (IDT, http://www.idtdna.com). The apple genome accession codes, gene description and primer sequences are shown in Supplementary Table S2. RT-qPCR was performed in a StepOnePlus Real-Time PCR System (Applied Biosystems). SYBR Green (Invitrogen, 1:100 dilution) was used to monitor dsDNA synthesis and ROX (Invitrogen, 1X) was employed as passive fluorescence reference. Each biological sample was analyzed in technical quadruplicates. Cycling protocol consisted of 95°C for 10 min, 40 cycles at 95°C for 15 s for denaturation and at 60°C for 1 min for annealing and extension followed by a dissociation curve between 60ºC and 95°C. The specificity of PCR amplifications was assessed by the presence of a single peak in melting curves and single bands of amplification products analyzed by 1.5% EtBr gel electrophoresis. Mean relative gene expression was calculated using the Pfaffl (2001) method with the *ARC5*, *MDH* and *WD40* genes as references (Perini et al. unpublished data). The parametric, two-tailed, unpaired Student's *t*-test was used to compare expression levels of samples from the same dates of different cultivars at Monte Castelo and Papanduva.

Supplementary data

Supplementary data are available at PCP online.

Funding

This work was supported by "Financiadora de Estudos e Projetos" (FINEP) and "Empresa Brasileira de Pesquisa Agropecuária" (EMBRAPA), Brazil. VSF received a M.Sc. scholarship from "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior" (CAPES, Ministry of Education, Brazil). GP and MMANPM are recipients of research fellowships from "Conselho Nacional de Desenvolvimento Científico e Tecnológico" (CNPq, Ministry of Science and Technology, Brazil; grant numbers 311361/2009-9 and 308708/2006-7).

Acknowledgments

We gratefully acknowledge our colleagues at Laboratory of Plant Molecular Genetics and Laboratory of Plant Physiology at EMBRAPA Grape and Wine, who helped us at many technical steps.

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Legend to Figures

Fig. 1 (a-d) Gene Ontology (GO) terms belonging to biological process domain attributed to unigene groups identified in SSH libraries. Dormant buds of 'Gala Standard' (G) or 'Castel Gala' (K) harvested in May (1) or August (2) at the city of Monte Castelo (SC) were used to construct four SSH libraries representing genes in dormancy entrance (G1-K1 and K1-G1), dormancy maintenance (G2-K2) and dormancy release (K2-G2). G1-K1 (a), G2-K2 (b), K1-G1 (c) and K2-G2 (d). GO terms were only attributed to groups of at least 10 unigenes. Number of unigenes annotated within each term is shown.

Fig. 2 RT-qPCR relative expression levels of 17 candidate genes identified by SSH, validating this screening technique. Subtractive cDNA libraries were constructed as described in the legend in Fig. 1. *RAP2.12* was identified by the G1-K1 subtraction. Both histones were identified by K2-G2, and HTA8 was also identified by G1-K1. The remaining genes were identified by the G2-K2, except *LTI65* that was also identified by G1-K1. RT-qPCR were performed with the 2007 original samples (used for SSH library construction) and also with two additional samples for both cultivars at Monte Castelo (see Materials and Methods). Relative transcript levels in 'Gala Standard' first sampling (G1) was set to 1. Standard error bars are shown. Asterisks indicate statistical significance between cultivars to the same sampling date (unpaired *t* test: ** $p<0.01$, * $p<0.05$).

Fig. 3 RT-qPCR relative expression levels of ten genes over 2009/2010. RT-qPCRs were performed in closed bud samples harvested from apple 'Royal Gala' and 'Castel Gala' trees grown in Papanduva (SC). Relative transcript levels in February 2010 sample of 'Royal Gala' was set to 1. Standard error bars are shown. Asterisks indicate statistical significance between cultivars to the same sampling point (unpaired *t* test: ** $p<0.01$, * $p<0.05$).

Fig. 4 RT-qPCR relative expression levels of ten genes over 2009/2010. RT-qPCRs were performed in closed bud samples harvested from apple 'Fuji Standard' trees grown in Caçador (SC). Relative transcript levels in February 2010 sample of 'Fuji Standard' was set to 1. Standard error bars are shown. 50% of buds in silver tip stage occurred in September 15th, 2009.

FIGURES

Fig. 1

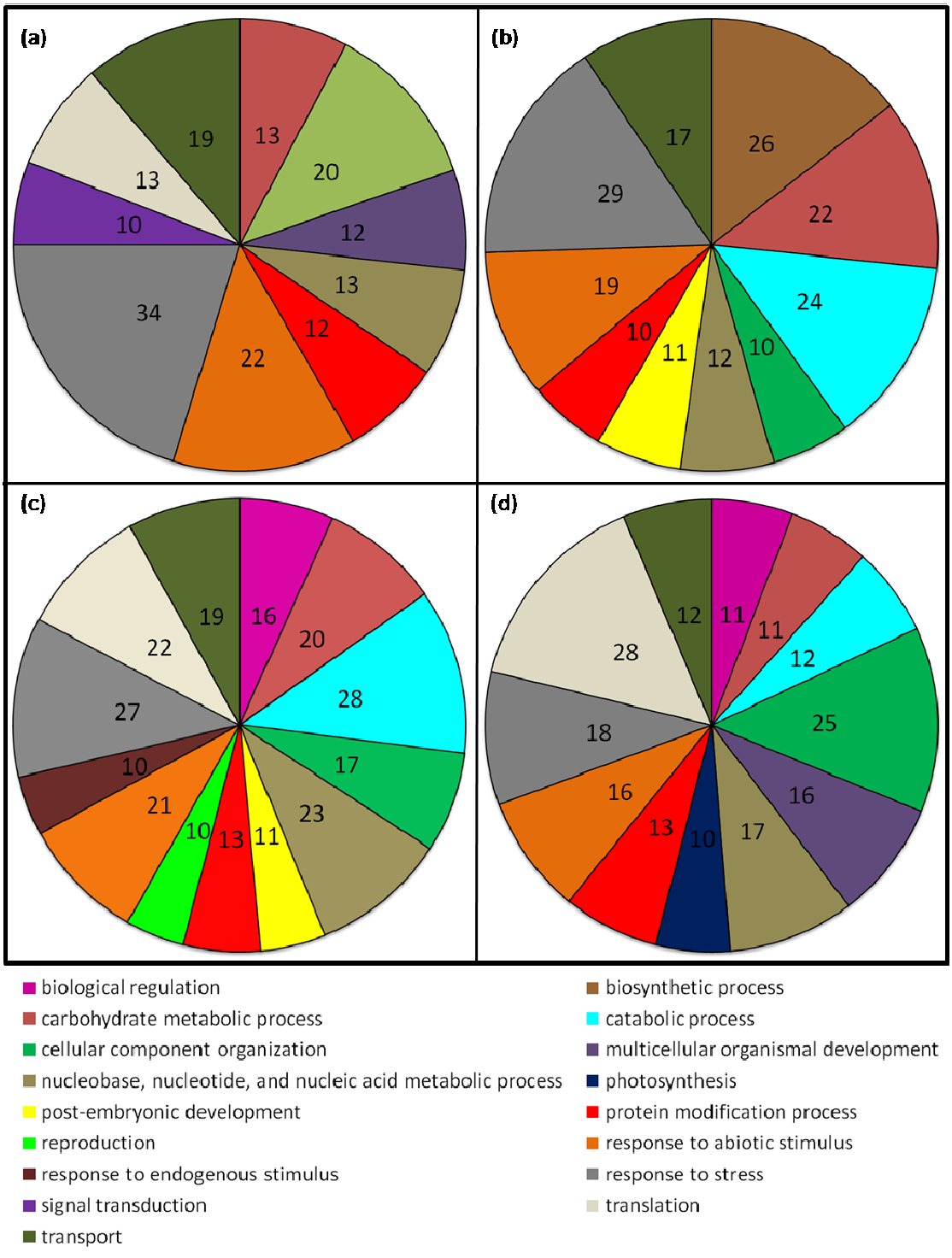
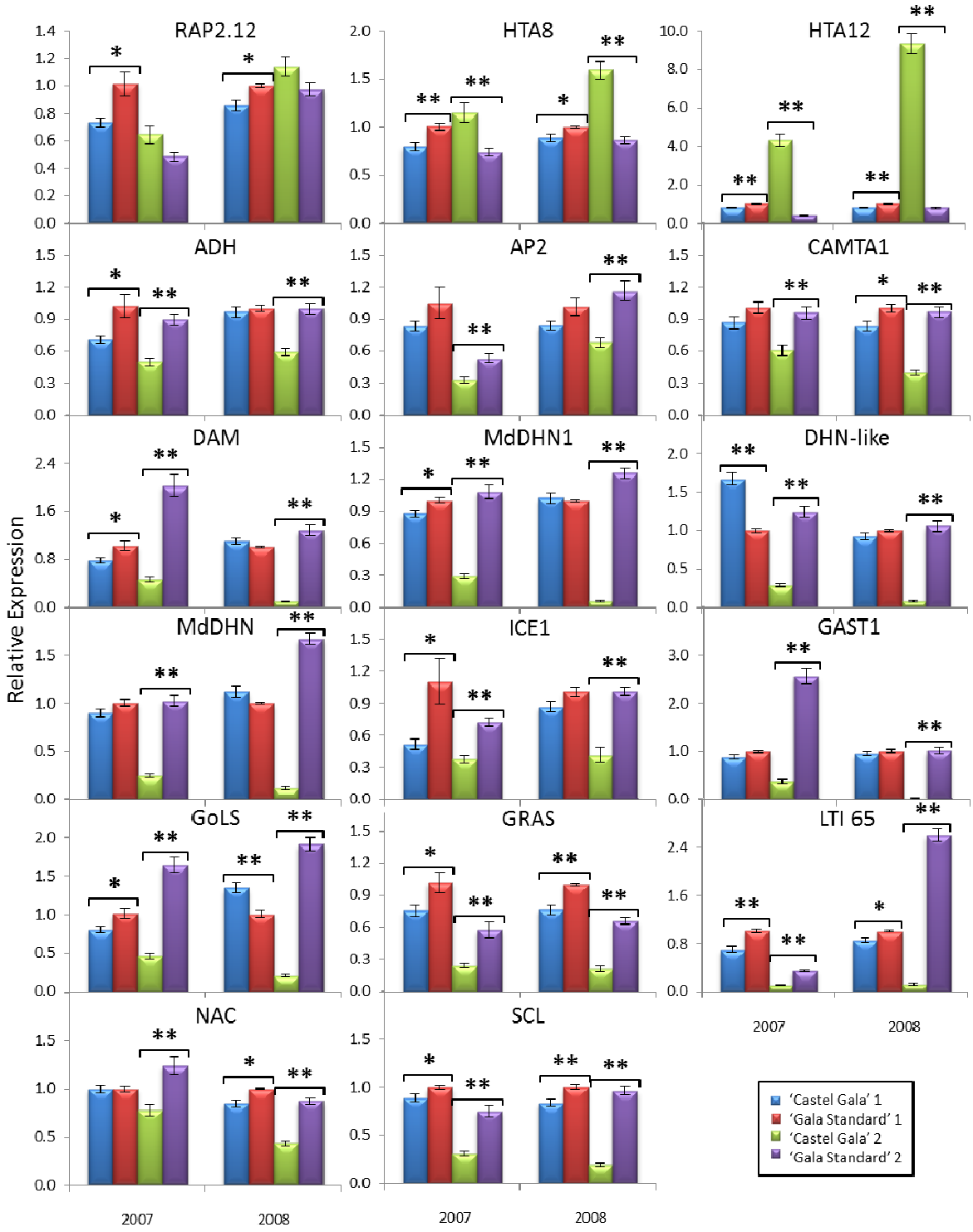


Fig. 2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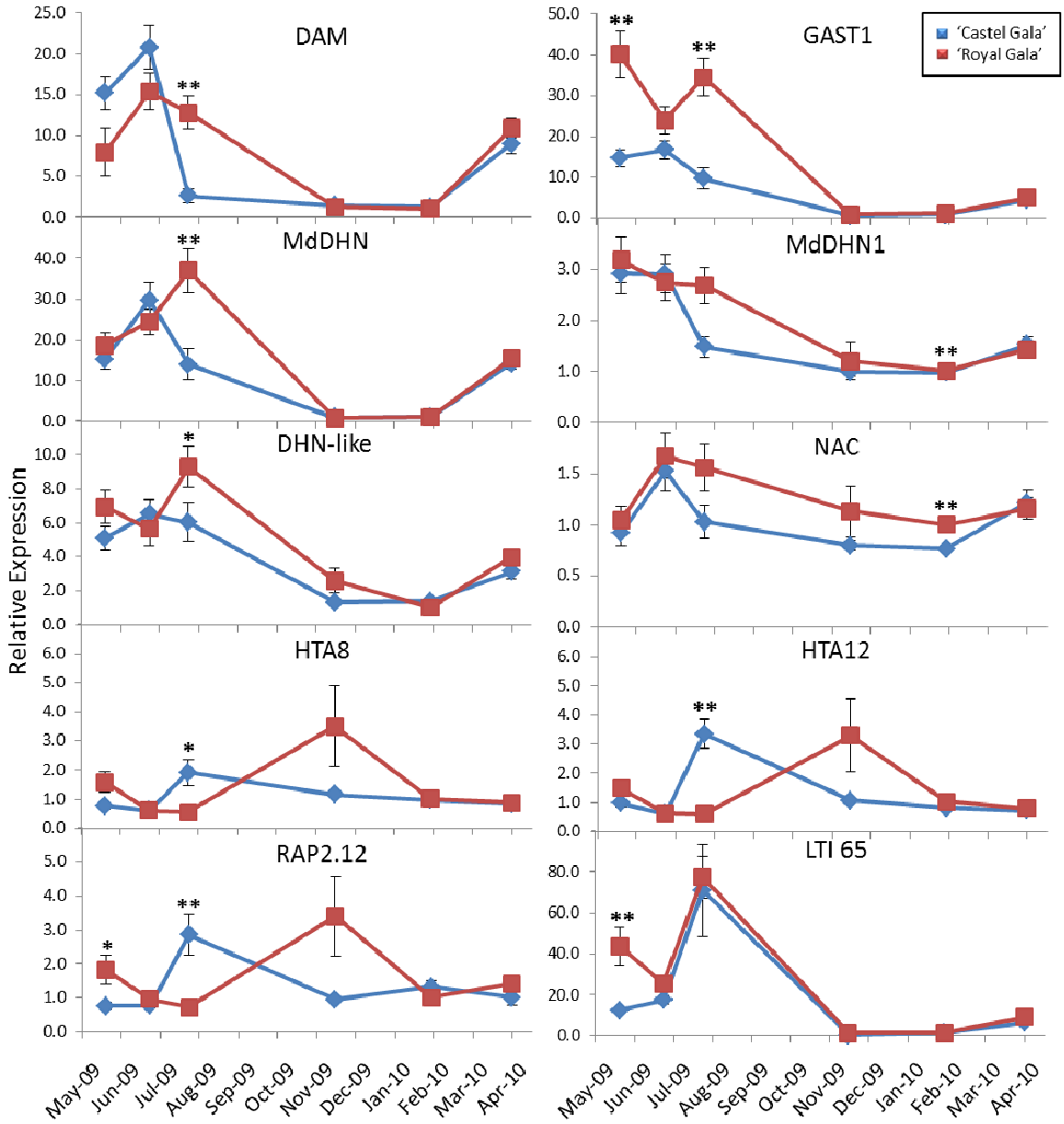
MdDHN
ICE1
GAST1
GoLS
GRAS
LTI 65
NAC
SCL
Relative Expression
2007
2008
'Castel Gala' 1
'Gala Standard' 1
'Castel Gala' 2
'Gala Standard' 2

Fig. 3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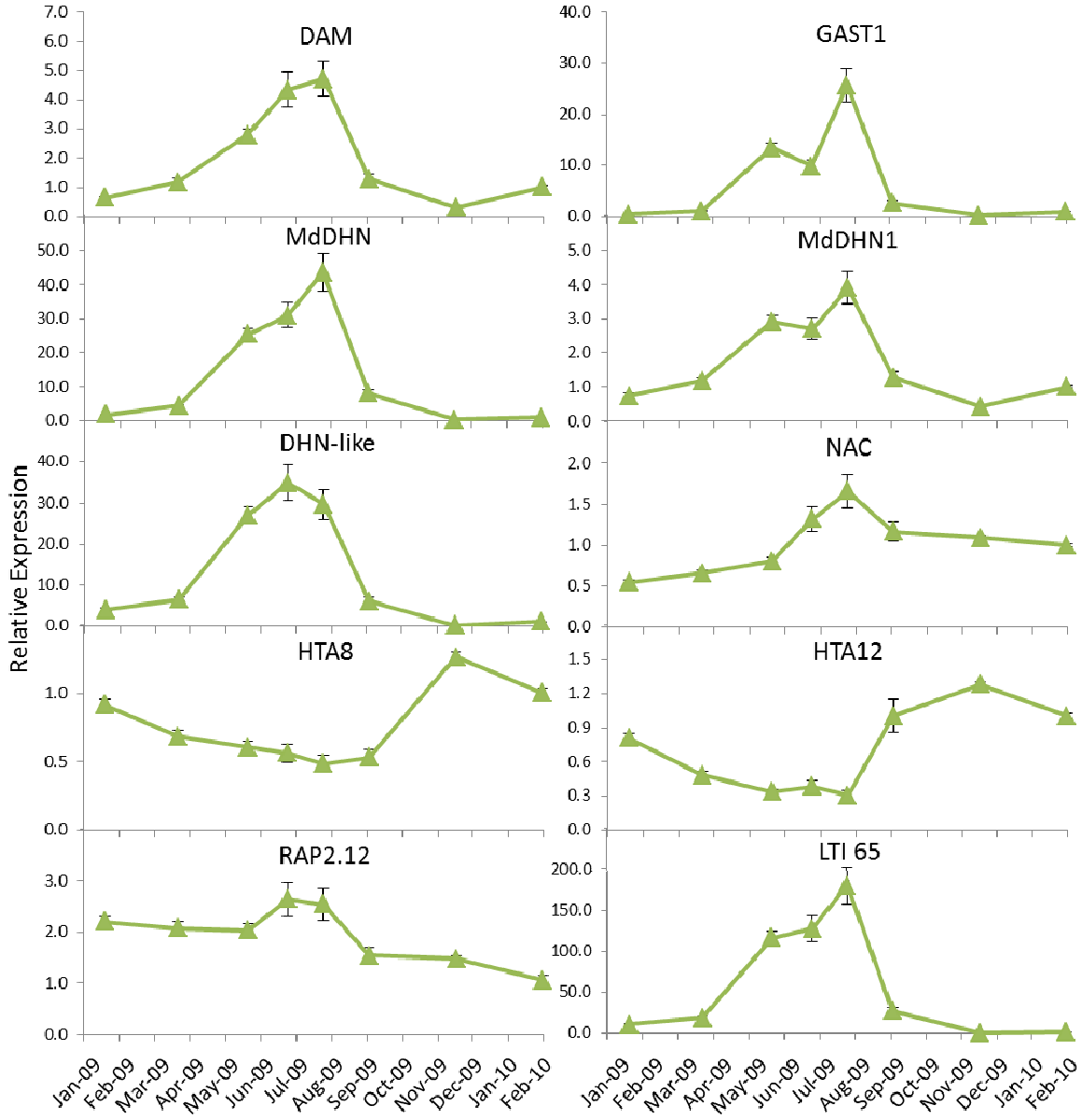
GAST1
MdDHN
MdDHN1
DHN-like
NAC
HTA8
HTA12
RAP2.12
LTI 65
'Castel Gala'
'Royal Gala'
Relative Expression
May-09
Jun-09
Jul-09
Aug-09
Sep-09
Oct-09
Nov-09
Dec-09
Jan-10
Feb-10
Mar-10
Apr-10

Fig.4


DAM
GAST1
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LTI 65
Relative Expression
Jan-09
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Apr-09
May-09
Jun-09
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Aug-09
Sep-09
Oct-09
Nov-09
Dec-09
Jan-10
Feb-10

TABLES

Table 1. Complementary DNA origins for SSH library construction. Dormant buds of 'Gala Standard' (G) or 'Castel Gala' (K) harvested in May (1) or August (2) in Monte Castelo (SC). Obtained SSH libraries represent genes in dormancy entrance (G1-K1 and K1-G1), dormancy maintenance (G2-K2) and dormancy release (K2-G2). In south Brazil, May is the 3rd autumn month and August is the 3rd winter month.

SSH library	**Tester**	**Driver**
G1-K1	'Gala Standard' (May)	'Castel Gala' (May)
G2-K2	'Gala Standard' (August)	'Castel Gala' (August)
K1-G1	'Castel Gala' (May)	'Gala Standard' (May)
K2-G2	'Castel Gala' (August)	'Gala Standard' (August)

Table 2. Significantly enriched ($p<0.05$) gene ontology classifications after projection to more generic GO terms related to plants (GO plant slim).

Library	GO term description	*p*-value	EST number
G1-K1	small molecule metabolic process	$1.23\ e^{-4}$	22
	cellular amino acid and derivative metabolic process	$1.23\ e^{-4}$	22
K1-G1	- not significant -	-	-
G2-K2	carbohydrate metabolic process	$3.33\ e^{-6}$	37
	response to stress	$6.79\ e^{-5}$	68
	response to abiotic stimulus	$2.71\ e^{-4}$	55
	response to stimulus	$1.88\ e^{-3}$	81
K2-G2	cell part	$5.73\ e^{-5}$	192
	cellular component organization	$1.14\ e^{-3}$	43
	membrane	$2.07\ e^{-3}$	88
	cellular process	$2.57\ e^{-3}$	162
	intracellular organelle	$2.73\ e^{-3}$	136
	organelle	$2.73\ e^{-3}$	136
	photosynthesis	$2.87\ e^{-3}$	15
	intracellular part	$3.02\ e^{-3}$	163
	lipid metabolic process	$4.49\ e^{-3}$	15

Table 3. Chilling hours accumulated by dormant buds harvested in 2009 and 2010. 'Fuji Standard' experimental area is located in Caçador (SC). 'Royal Gala' and 'Castel Gala' experimental area belongs to a commercial orchard at the city of Papanduva (SC).

Caçador (SC)		**Papanduva (SC)**	
Date	**Hours below 7.2°C**	**Date**	**Hours below 7.2°C**
01/21/2009	0		
03/26/2009	0		
05/27/2009	76	05/26/2009	53
06/30/2009	278	06/30/2009	282
07/30/2009	450	07/30/2009	446
09/09/2009	528		
11/25/2009	528	11/24/2009	578
02/01/2010	0	02/08/2010	0
		04/13/2010	0

SUPPLEMENTARY DATA

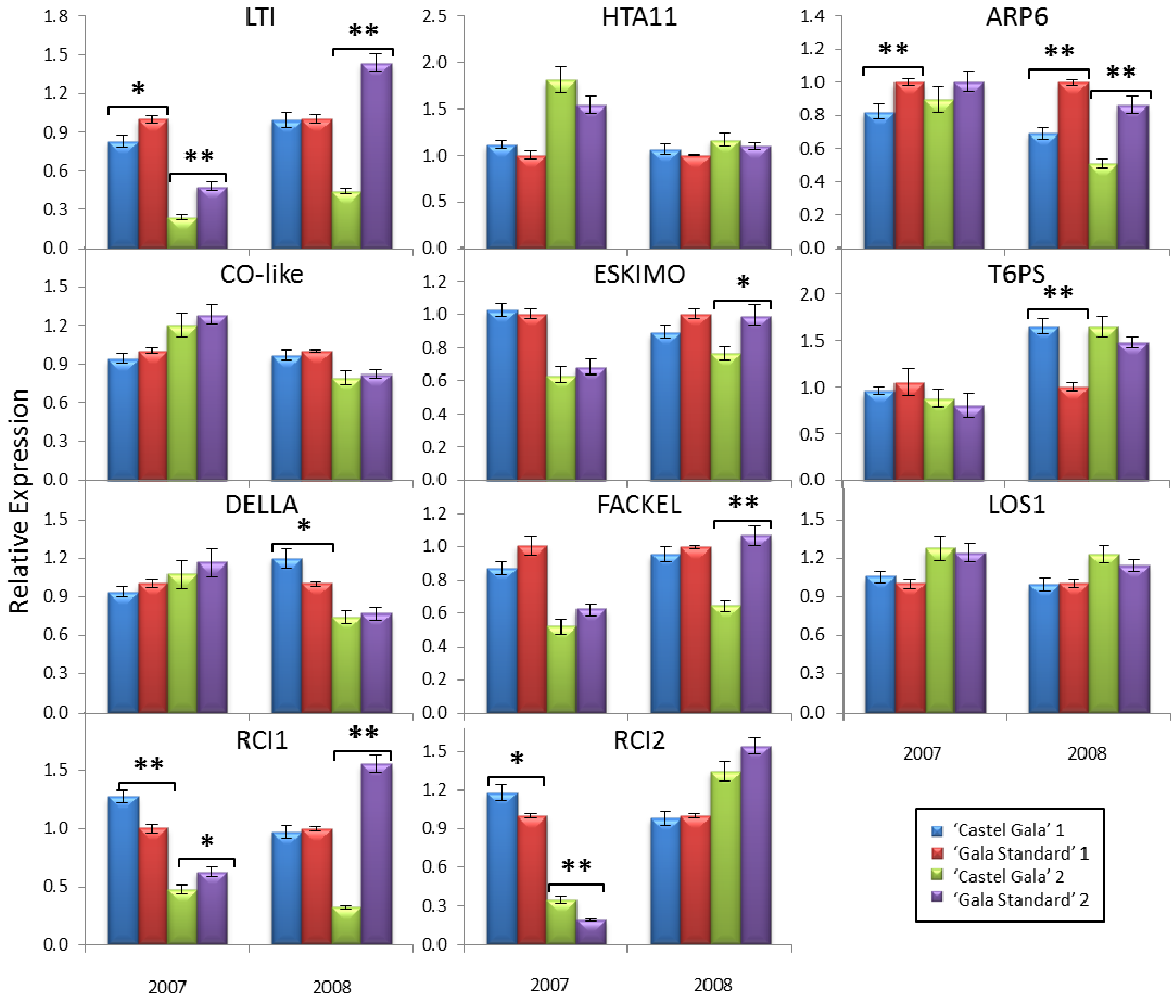
Supplementary Fig. S1 RT-qPCR relative expression levels of 11 candidate genes identified by SSH, validating this screening technique. Subtractive cDNA libraries were constructed as described in the legend in Fig. 1. *LTI* and HTA11 were identified by G1-K1 subtraction. *ARP6*, CO-like, ESKIMO and T6PS were identified by G2-K2. The remaining genes were identified by K1-G1. RT-qPCR were performed with the 2007 original samples (used for SSH library construction) and also with two additional samples for both cultivars at Monte Castelo (see Materials and Methods). Relative transcript levels for 'Gala Standard' first sampling (G1) was set to 1. Standard error bars are shown. Asterisks indicate statistical significance between cultivars to the same sampling date (unpaired *t* test: ** $p < 0.01$, * $p < 0.05$).

Supplementary Fig. S2 RT-qPCR relative expression levels of seven genes over 2009/2010. RT-qPCRs were performed in closed bud samples harvested from apple 'Royal Gala' and 'Castel Gala' trees grown in Papanduva (SC). The genes depicted here have not shown the same SSH pattern in May and July samples of 2009. Relative transcript levels in February 2010 sample of 'Royal Gala' were set to 1. Standard error bars are shown.

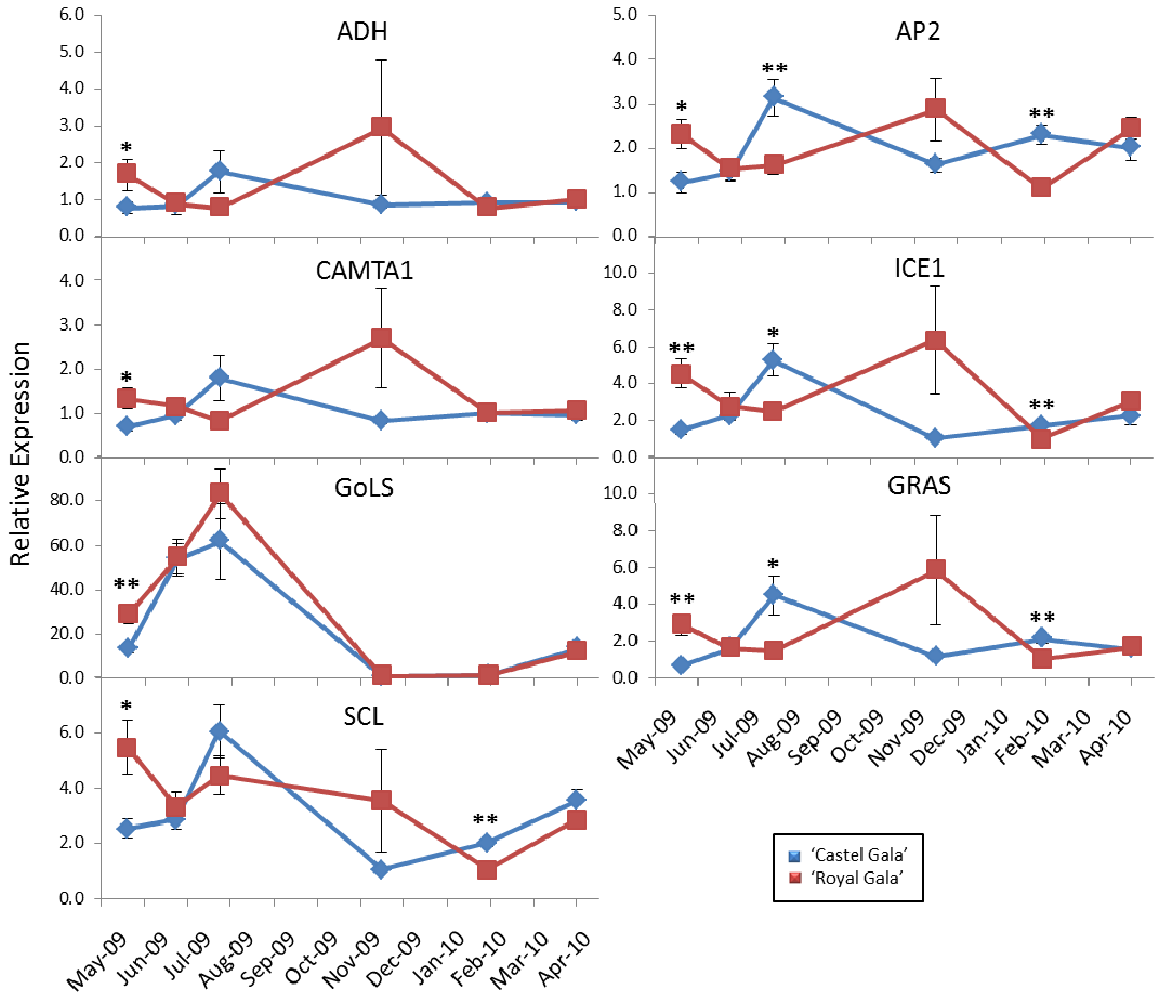
Supplementary Fig. S3 RT-qPCR relative expression levels of seven genes over 2009/2010. RT-qPCRs were performed in closed bud samples harvested from apple 'Fuji Standard' trees grown in Caçador (SC). Relative transcript levels in February 2010 sample of 'Fuji Standard' was set to 1. Standard error bars are shown. 50% of buds in silver tip stage occurred in September 15th, 2009.

SUPPLEMENTARY FIGURES

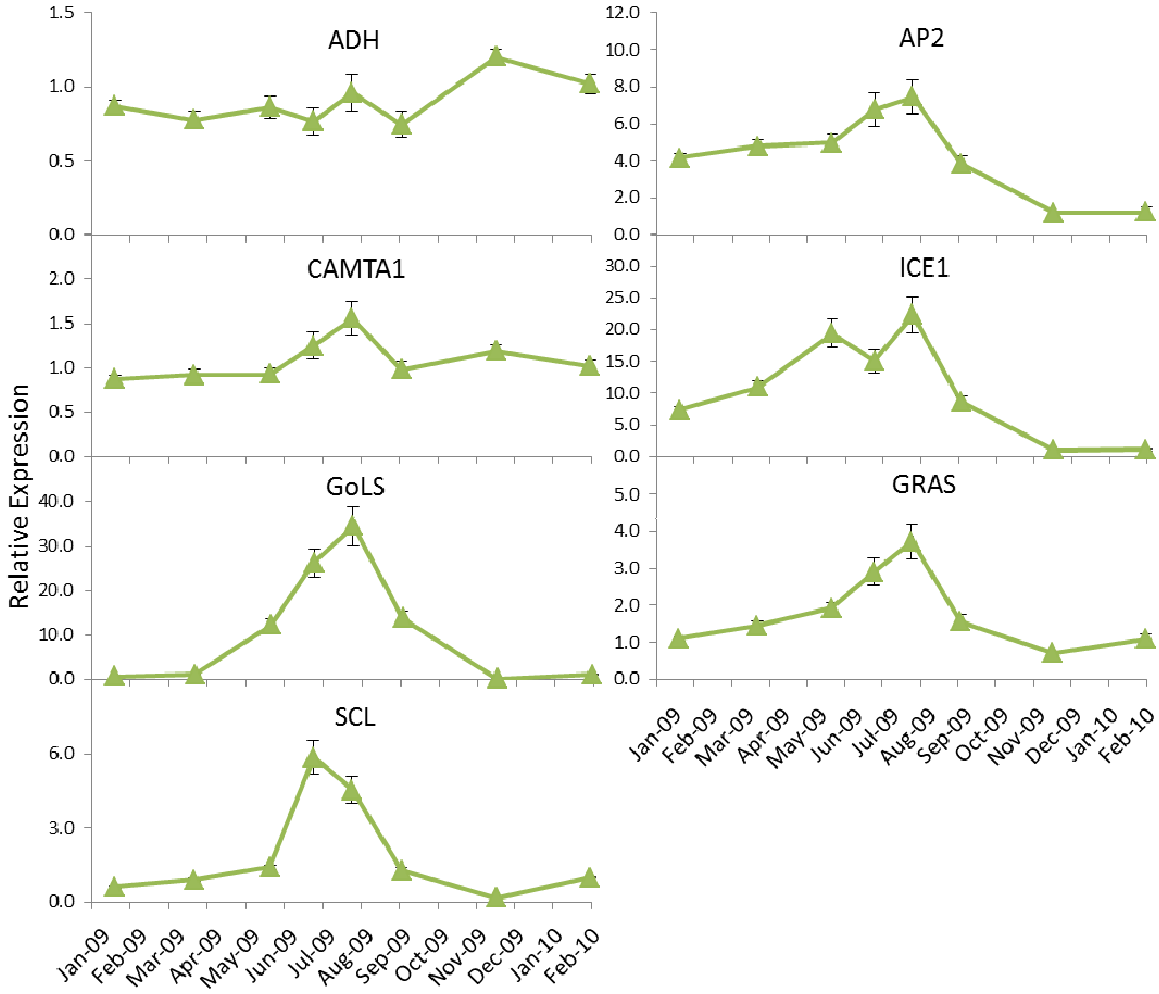
Supplementary Fig. S1



Supplementary Fig. S2



Supplementary Fig. S3



Supplementary Table S1. Assembled sequences and singlets derived from SSH libraries of 'Gala Standard' vs 'Castel Gala' bud apple cultivars. Sequence lengths (bp), best BlastX hits and significant GO terms attributed are indicated, as well as associated data. GO terms are classified in three general domains: molecular function (F), cellular component (C) and biological process (P).

Library	Sequence ID	Sequence Length (bp)	Best BlastX Hit	Minimum eValue	Mean Similarity	Gene Ontology Terms
G1-K1	Mado-00-G1-K1-Contig1	536	metallothionein-like protein	5.67388E-26	88.30%	F:metal ion binding
	Mado-00-G1-K1-Contig4	282	dehydrin-like protein	1.1197E-27	75.25%	P:response to water; P:response to stress
	Mado-00-G1-K1-Contig5	434	40s ribosomal protein s21	4.72904E-31	92.25%	F:structural constituent of ribosome; P:ribosome biogenesis; C:cytosolic small ribosomal subunit; P:translation
	Mado-00-G1-K1-Contig6	350	lipid transfer protein	1.60128E-18	67.15%	F:heme binding; P:lipid transport; P:oxidation reduction; F:monooxygenase activity; F:electron carrier activity; F:lipid binding
	Mado-00-G1-K1-Contig8	544	plastid jasmonates zim-domain protein	4.15056E-43	58.55%	P:response to wounding; F:protein binding; P:response to jasmonic acid stimulus
	Mado-00-G1-K1-Contig9	497	unknown [Populus trichocarpa]	3.71182E-60	81.30%	C:anchored to membrane; C:cytoplasmic membrane-bounded vesicle; C:plasma membrane
	Mado-00-G1-K1-Contig10	484	chalcone isomerase	2.30434E-41	89.25%	P:flavonoid biosynthetic process; F:chalcone isomerase activity
	Mado-00-G1-K1-Contig11	468	high mobility group family	1.25446E-39	77.70%	P:DNA repair; P:oxidation reduction; P:regulation of transcription; P:DNA replication; F:2-alkenal reductase activity; C:nuclear euchromatin; F:transcription factor activity; C:FACT complex
	Mado-00-G1-K1-Contig12	458	zinc finger	5.38183E-35	67.30%	F:hydrolase activity, acting on ester bonds; C:cytoplasmic membrane-bounded vesicle; C:mitochondrion
	Mado-00-G1-K1-Contig13	444	pre-mrna-processing atp-dependent rna helicase prp-5	7.43661E-16	72.75%	P:auxin biosynthetic process; F:ATP-dependent helicase activity; F:nucleic acid binding; F:ATP binding
	Mado-00-G1-K1-Contig14	442	protein	1.70992E-36	79.80%	C:chloroplast thylakoid membrane
	Mado-00-G1-K1-Contig15	438	cysteine protease	1.10308E-72	88.60%	C:endomembrane system; P:aging; P:proteolysis; F:protein binding; P:response to ethylene stimulus; F:cysteine-type endopeptidase activity
	Mado-00-G1-K1-Contig16	411	proteasome subunit alpha type 7	8.30488E-36	94.95%	P:flower development; C:vacuole; P:defense response to bacterium, incompatible interaction; F:threonine-type endopeptidase activity; P:response to jasmonic acid stimulus; P:defense response to fungus; P:ubiquitin-dependent protein catabolic process; P:indole phytoalexin biosynthetic process; C:proteasome core complex, alpha-subunit complex; C:chloroplast stroma; P:callose deposition in cell wall during defense response; P:glucosinolate biosynthetic process; P:defense response to insect; P:response to cadmium ion; P:response to heat; P:response to ozone; P:glutathione biosynthetic process; C:nucleus; F:glutamate-cysteine ligase activity
	Mado-00-G1-K1-Contig17	407	ubiquitin conjugating	2.9923E-17	67.90%	P:protein metabolic process; F:small conjugating protein ligase activity
	Mado-00-G1-K1-Contig18	353	mitochondrial serine hydroxymethyltransferase	2.78959E-47	95.10%	F:pyridoxal phosphate binding; P:L-serine metabolic process; P:one-carbon metabolic process; F:glycine hydroxymethyltransferase activity; P:glycine metabolic process; C:mitochondrion
	Mado-00-G1-K1-Contig20	335	precursor of transferase serine hydroxymethyltransferase 3	2.26633E-33	82.30%	F:pyridoxal phosphate binding; P:L-serine metabolic process; P:one-carbon metabolic process; F:glycine hydroxymethyltransferase activity; P:glycine metabolic process; C:mitochondrion
	Mado-00-G1-K1-Contig21	322	peroxidase prxr1	1.14456E-24	97.10%	F:heme binding; P:oxidation reduction; F:peroxidase activity; F:protein binding; P:response to oxidative stress
	Mado-00-G1-K1-Contig23	270	low-temperature inducible	7.62012E-13	66.95%	C:chloroplast envelope; P:response to cold; P:response to bacterium; P:response to cadmium ion; C:plasma membrane; C:chloroplast stroma; P:response to heat; P:response to salt stress; P:abscisic acid mediated signaling pathway
	Mado-00-G1-K1-Contig24	232	protein phosphatase	1.55108E-5	77.86%	P:protein amino acid dephosphorylation; F:metal ion binding; F:protein serine/threonine phosphatase activity; C:protein serine/threonine phosphatase complex
	Mado-00-G1-K1-Contig25	231	protein phosphatase regulatory	1.30094E-36	95.55%	C:protein phosphatase type 2A complex; P:signal transduction; C:chloroplast; F:protein phosphatase type 2A regulator activity
	Mado-00-G1-K1-Contig27	221	unnamed protein product [Vitis vinifera]	3.74213E-7	69.28%	F:protein binding; P:apoptosis
	Mado-00-G1-K1-Contig28	202	ketoacyl-acp reductase	2.62064E-21	94.90%	P:fatty acid biosynthetic process; F:NAD or NADH binding; F:3-oxoacyl-[acyl-carrier-protein] reductase activity; P:oxidation reduction; C:chloroplast
	Mado-00-G1-K1-Contig29	183	40s ribosomal protein s5	2.96179E-12	82.05%	F:structural constituent of ribosome; C:plasma membrane; F:RNA binding; C:cell wall; C:cytosolic small ribosomal subunit; C:chloroplast; P:translation
	Mado-00-G1-K1-Contig33	140	ran-family small gtpase	2.7656E-15	100.00%	F:GTP binding; F:protein binding; P:nucleocytoplasmic transport; P:intracellular protein transport; P:signal transduction; F:GTPase activity
	Mado-00-G1-K1-001_A04	189	serine threonine-protein kinase	8.01343E-10	77.45%	P:protein amino acid phosphorylation; F:ATP binding; F:protein serine/threonine kinase activity
	Mado-00-G1-K1-001_A05	112	60s ribosomal protein l21	5.36949E-14	100.00%	C:ribosome; F:structural constituent of ribosome; P:translation; C:mitochondrion
	Mado-00-G1-K1-001_A06	108	ceramidase family protein	1.56701E-13	87.20%	C:endomembrane system; F:ceramidase activity
	Mado-00-G1-K1-001_A07	186	xyloglucan endotransglycosylase	1.11664E-27	96.55%	F:hydrolase activity, hydrolyzing O-glycosyl compounds; P:cellular glucan metabolic process; C:apoplast; C:cell wall; F:xyloglucan:xyloglucosyl transferase activity
	Mado-00-G1-K1-001_A09	305	cyclophilin	1.12697E-32	94.85%	F:peptidyl-prolyl cis-trans isomerase activity; P:protein folding
	Mado-00-G1-K1-001_A11	213	dna topoisomerase iii	1.42228E-11	81.90%	P:DNA unwinding involved in replication; P:DNA repair; F:zinc ion binding; P:resolution of meiotic recombination intermediates; F:DNA topoisomerase type I activity; P:mitosis; C:chromosome; P:DNA topological change
	Mado-00-G1-K1-001_A12	507	protein	2.1411E-26	79.85%	C:endomembrane system; C:cytoplasmic membrane-bounded vesicle; C:membrane
	Mado-00-G1-K1-001_B03	142	chalcone synthase	7.02122E-19	100.00%	F:naringenin-chalcone synthase activity; P:biosynthetic process; F:acyltransferase activity
	Mado-00-G1-K1-001_B05	151	dimethylaniline	8.01114E-18	87.55%	F:flavin-containing monooxygenase activity; F:NADP or NADPH binding; P:oxidation reduction; C:intrinsic to endoplasmic reticulum membrane; F:FAD binding
	Mado-00-G1-K1-001_B07	247	protein	1.51409E-29	94.60%	P:cytoskeleton organization; P:protein amino acid phosphorylation; C:vacuole; P:auxin biosynthetic process; F:protein kinase activity; F:ATP:ADP antiporter activity; C:mitochondrial inner membrane; C:integral to membrane; C:chloroplast; C:cell wall; P:root hair elongation; C:nucleolus; C:cytoskeleton; P:purine nucleotide transport; P:transmembrane transport; C:plasma membrane; F:copper ion binding; F:ATP binding; F:structural constituent of cytoskeleton; P:mitochondrial transport
	Mado-00-G1-K1-001_B08	298	acetylornithine transaminase	5.27354E-30	85.05%	F:copper ion binding; P:response to water deprivation; C:mitochondrion; P:arginine biosynthetic process; P:defense response to bacterium; P:regulation of transcription, DNA-dependent; F:N2-acetyl-L-ornithine:2-oxoglutarate 5-aminotransferase activity; P:cutin biosynthetic process; F:sequence-specific DNA binding; F:transcription factor activity; C:chloroplast stroma; P:wax metabolic process; F:pyridoxal phosphate binding; C:nucleus; P:ethylene mediated signaling pathway
	Mado-00-G1-K1-001_B10	111	chlorophyll a b-binding protein cp29	3.25755E-11	91.00%	F:protein binding
	Mado-00-G1-K1-001_B11	262	at3g26650 mlj15_5	5.85581E-37	91.45%	F:glyceraldehyde-3-phosphate dehydrogenase (phosphorylating) activity; P:reductive pentose-phosphate cycle; P:response to sucrose stimulus; F:glyceraldehyde-3-phosphate dehydrogenase (NADP+) (phosphorylating) activity; P:response to light stimulus; P:response to cold; P:oxidation reduction; F:NAD or NADH binding; F:protein binding; P:glycolysis; C:chloroplast stroma; C:membrane; C:stromule; C:apoplast
	Mado-00-G1-K1-001_B12	308	60s ribosomal protein l7a	6.78039E-46	95.05%	C:ribosome; F:binding; F:structural constituent of ribosome; P:ribosome biogenesis; P:translation
	Mado-00-G1-K1-001_C01	118	diacylglycerol kinase	6.52403E-12	89.65%	P:intracellular signaling pathway; P:activation of protein kinase C activity by G-protein coupled receptor protein signaling pathway; F:calcium ion binding; F:diacylglycerol kinase activity
	Mado-00-G1-K1-001_C03	150	protein	7.0181E-14	75.30%	C:vacuole
	Mado-00-G1-K1-001_C04	265	cystathionine gamma-synthase	6.02453E-42	92.25%	F:pyridoxal phosphate binding; F:cystathionine gamma-synthase activity; P:methionine biosynthetic process; F:lyase activity; C:chloroplast
	Mado-00-G1-K1-001_C05	118	glycine rich protein	4.67544E-10	82.60%	P:response to water; P:response to stress
	Mado-00-G1-K1-001_C06	154	pyruvate dehydrogenase kinase	5.17637E-17	92.95%	F:histidine phosphotransfer kinase activity; F:two-component sensor activity; P:peptidyl-histidine phosphorylation; F:ATP binding; P:signal transduction; F:pyruvate dehydrogenase (acetyl-transferring) kinase activity; C:mitochondrion
	Mado-00-G1-K1-001_C08	232	binding protein	1.76236E-25	78.92%	F:DNA binding; P:DNA integration
	Mado-00-G1-K1-001_C11	200	auxin-repressed protein	5.48244E-19	92.50%	P:auxin mediated signaling pathway; F:lyase activity

Mado-00-G1-K1-001_C12	241	mandelonitrile lyase	2.24594E-30	83.85%	F:FAD binding; C:cytoplasmic membrane-bounded vesicle; F:oxidoreductase activity, acting on CH-OH group of donors; P:alcohol metabolic process; F:lyase activity
Mado-00-G1-K1-001_D02	240	heat shock protein 101	6.61315E-33	90.55%	P:auxin biosynthetic process; P:response to high light intensity; F:ATPase activity; F:protein binding; F:ATP binding; P:response to hydrogen peroxide; P:protein unfolding; P:response to heat
Mado-00-G1-K1-001_D07	203	grf6 (g-box regulating factor 6) protein binding protein phosphorylated amino acid binding	2.07165E-26	93.30%	P:defense response to bacterium; C:plant-type cell wall; F:protein domain specific binding; P:response to cadmium ion; C:plasma membrane; F:protein phosphorylated amino acid binding; C:chloroplast; C:nucleus; C:cytosol; P:brassinosteroid mediated signaling pathway
Mado-00-G1-K1-001_D09	231	phosphoinositide phosphatase family protein	6.48652E-20	64.73%	F:phosphatidylinositol-4,5-bisphosphate 5-phosphatase activity; P:biological_process; C:cellular_component
Mado-00-G1-K1-001_D11	303	ubiquitin carboxyl-terminal	2.43739E-19	83.20%	F:ubiquitin-specific protease activity; P:ubiquitin-dependent protein catabolic process; F:ubiquitin thiolesterase activity; C:mitochondrion
Mado-00-G1-K1-001_E04	213	protein	3.26371E-24	82.00%	F:transferase activity; C:mitochondrion
Mado-00-G1-K1-001_F06	170	protein	3.5283E-18	78.80%	P:regulation of transcription; C:anchored to membrane; F:transcription factor activity; F:zinc ion binding; C:plastid
Mado-00-G1-K1-001_F07	333	flavonoid 3-	2.12122E-31	83.10%	F:heme binding; F:flavonoid 3',5'-hydroxylase activity; P:oxidation reduction; F:oxidoreductase activity, acting on single donors with incorporation of molecular oxygen, incorporation of two atoms of oxygen; F:electron carrier activity
Mado-00-G1-K1-001_F08	274	kinase family protein	2.67568E-42	94.45%	P:auxin biosynthetic process; P:protein amino acid phosphorylation; F:ATP binding; F:protein serine/threonine kinase activity
Mado-00-G1-K1-001_F09	246	protein	5.22804E-14	71.63%	P:response to stress
Mado-00-G1-K1-001_G01	130	protein	1.38737E-14	91.25%	C:cytoplasm; F:proline-tRNA ligase activity; P:prolyl-tRNA aminoacylation; F:protein binding; F:ATP binding; C:membrane
Mado-00-G1-K1-001_G03	212	geranylgeranyl reductase	1.68571E-12	93.70%	P:geranylgeranyl diphosphate metabolic process; P:phytyl diphosphate biosynthetic process; P:oxidation reduction; P:vitamin E biosynthetic process; P:tRNA processing; P:chlorophyll biosynthetic process; F:monooxygenase activity; F:protein binding; P:photosynthesis; F:FAD binding; C:chloroplast; F:geranylgeranyl reductase activity
Mado-00-G1-K1-001_G06	339	signal recognition particle subunit	1.15508E-29	69.85%	F:DNA binding; C:cytoplasmic membrane-bounded vesicle; P:SRP-dependent cotranslational protein targeting to membrane; P:DNA integration; C:signal recognition particle; F:7S RNA binding
Mado-00-G1-K1-001_G07	348	protein	2.00227E-53	89.30%	C:Golgi apparatus
Mado-00-G1-K1-001_G11	275	protein kinase	2.5856E-45	96.55%	P:auxin biosynthetic process; P:protein amino acid phosphorylation; F:non-membrane spanning protein tyrosine kinase activity; P:defense response to insect; P:defense response to fungus; F:ATP binding; F:protein serine/threonine kinase activity; C:plastid
Mado-00-G1-K1-001_H01	151	beta-galactosidase like protein	5.36112E-22	87.65%	P:carbohydrate metabolic process; C:cytoplasmic membrane-bounded vesicle; F:protein binding; F:beta-galactosidase activity; F:sugar binding; F:cation binding
Mado-00-G1-K1-001_H04	141	dt-related protein	1.84769E-19	90.95%	P:metabolic process; F:phosphoglycolate phosphatase activity; C:mitochondrion
Mado-00-G1-K1-001_H08	237	protein	8.39445E-28	78.10%	C:endomembrane system; C:membrane; C:cytoplasmic membrane-bounded vesicle
Mado-00-G1-K1-002_A06	403	outward rectifying potassium channel	4.43815E-53	90.95%	C:plant-type vacuole membrane; F:calcium ion binding; P:potassium ion transport; C:integral to membrane; F:outward rectifier potassium channel activity
Mado-00-G1-K1-002_A07	117	lipid transfer protein	1.96433E-8	79.60%	F:lipid binding; P:lipid transport
Mado-00-G1-K1-002_A08	261	1-aminocyclopropane-1-carboxylate oxidase	1.87939E-43	96.95%	F:metal ion binding; P:ethylene biosynthetic process; P:ripening; F:L-ascorbic acid binding; F:obs-aminocyclopropane-1-carboxylate oxidase activity; P:oxidation reduction
Mado-00-G1-K1-002_A11	362	dna-directed rna polymerases and iii kda polypeptide	6.17072E-31	99.40%	F:DNA-directed RNA polymerase activity; P:transcription, DNA-dependent; C:RNA polymerase complex; F:DNA binding
Mado-00-G1-K1-002_A12	339	hexose transporter	4.87552E-12	82.65%	P:transmembrane transport; P:oxidation reduction; F:sugar:hydrogen symporter activity; P:carbohydrate transport; C:integral to membrane; F:2-alkenal reductase activity
Mado-00-G1-K1-002_B01	186	enolase	1.365E-25	97.20%	C:cell surface; C:phosphopyruvate hydratase complex; F:phosphopyruvate hydratase activity; F:hydrolase activity; F:magnesium ion binding; P:glycolysis
Mado-00-G1-K1-002_B04	315	glycine-rich rna-binding protein	1.11174E-35	93.25%	F:nucleic acid binding; F:nucleotide binding
Mado-00-G1-K1-002_B05	237	phospholipase d alpha	5.79987E-37	96.05%	P:lipid catabolic process; F:NAPE-specific phospholipase D activity; F:calcium ion binding; F:phospholipase D activity; C:membrane; P:phosphatidylcholine metabolic process
Mado-00-G1-K1-002_B06	136	plasma membrane intrinsic protein	8.08343E-15	95.30%	P:transmembrane transport; C:integral to membrane; F:transporter activity
Mado-00-G1-K1-002_B10	267	enolase	1.47866E-40	95.25%	F:copper ion binding; P:response to salt stress; P:response to light stimulus; P:response to cold; C:phosphopyruvate hydratase complex; C:cell surface; P:response to abscisic acid stimulus; F:magnesium ion binding; P:glycolysis; C:mitochondrial envelope; C:chloroplast; F:phosphopyruvate hydratase activity; C:nucleus; C:plasma membrane; C:apoplast
Mado-00-G1-K1-002_C01	537	predicted protein [Populus trichocarpa]	1.52714E-18	72.67%	F:molecular_function; P:biological_process; C:cellular_component
Mado-00-G1-K1-002_C02	147	50s ribosomal protein chloroplast	1.08614E-14	95.65%	C:ribosome; F:structural constituent of ribosome; C:chloroplast; C:membrane; P:translation
Mado-00-G1-K1-002_C03	430	protein	2.35705E-30	89.70%	P:pollen tube development; P:galactose metabolic process; P:embryonic development ending in seed dormancy; F:UDP-arabinose 4-epimerase activity; F:UDP-glucose 4-epimerase activity; F:coenzyme binding; P:nucleotide-sugar metabolic process; P:plant-type cell wall biogenesis; P:arabinose biosynthetic process; C:Golgi apparatus
Mado-00-G1-K1-002_C08	273	protein	2.42005E-43	92.70%	F:pyridoxal phosphate binding; F:obs-aminocyclopropane-1-carboxylate synthase activity; P:biosynthetic process; F:L-aspartate:2-oxoglutarate aminotransferase activity; C:plastid
Mado-00-G1-K1-002_D05	131	mal d	3.30218E-16	98.35%	P:response to biotic stimulus; P:defense response
Mado-00-G1-K1-002_D09	147	zinc-finger protein	2.04836E-13	82.05%	F:zinc ion binding; C:intracellular; F:protein binding
Mado-00-G1-K1-002_D10	351	nucleoid dna-binding-like protein	4.31371E-40	90.10%	C:plant-type cell wall; C:apoplast; P:proteolysis; F:aspartic-type endopeptidase activity; C:chloroplast
Mado-00-G1-K1-002_D11	128	at5g51550 k17n15_10	3.66224E-15	93.80%	C:plant-type cell wall
Mado-00-G1-K1-002_E01	275	two-pore calcium channel	4.00755E-30	87.15%	P:transmembrane transport; P:defense response; P:seed germination; F:calcium ion binding; C:integral to membrane; C:plant-type vacuole; F:voltage-gated calcium channel activity; P:regulation of stomatal movement; C:vacuolar membrane; P:calcium ion transport; P:calcium-mediated signaling; C:plasma membrane
Mado-00-G1-K1-002_E03	385	chaperone protein	3.97983E-54	95.70%	C:cytoplasm; F:metal ion binding; P:protein folding; C:plasma membrane; F:heat shock protein binding; F:ATP binding; F:unfolded protein binding; P:response to heat
Mado-00-G1-K1-002_E06	198	neutral invertase	1.25806E-31	98.20%	F:beta-fructofuranosidase activity; P:metabolic process; C:mitochondrion
Mado-00-G1-K1-002_E08	394	60s ribosomal protein l35	1.35911E-33	94.70%	C:nucleolus; F:structural constituent of ribosome; C:plasma membrane; P:ribosome biogenesis; C:cytosolic large ribosomal subunit; P:translation
Mado-00-G1-K1-002_E09	211	sucrose synthase	5.41019E-19	93.05%	P:suspensor development; P:sucrose biosynthetic process; F:sucrose synthase activity
Mado-00-G1-K1-002_E10	260	histone h2a	1.72171E-12	94.10%	P:defense response to bacterium; C:nucleosome; F:DNA binding; P:nucleosome assembly; F:protein binding; P:regulation of flower development; C:nucleus
Mado-00-G1-K1-002_E11	411	40s ribosomal protein s7	3.71064E-52	93.50%	C:ribosome; F:structural constituent of ribosome; P:translation
Mado-00-G1-K1-002_E12	330	rare cold inducible protein	4.92023E-20	91.65%	P:response to abscisic acid stimulus; C:integral to membrane; P:response to cold; P:hyperosmotic salinity response
Mado-00-G1-K1-002_F02	450	metal tolerance protein	5.77391E-53	95.30%	P:cellular zinc ion homeostasis; C:plasma membrane; P:zinc ion transport; P:transmembrane transport; C:vacuolar membrane; P:oxidation reduction; F:inorganic anion transmembrane transporter activity; P:response to metal ion; F:zinc ion transmembrane transporter activity; F:2-alkenal reductase activity
Mado-00-G1-K1-002_F03	315	pif-like orf1	8.84981E-17	82.33%	C:ribosome; F:structural constituent of ribosome; P:translation; C:mitochondrion
Mado-00-G1-K1-002_F06	155	conserved hypothetical protein [Ricinus communis]	2.48828E-11	79.50%	C:plasma membrane
Mado-00-G1-K1-002_F09	273	cysteine protease	1.62328E-39	88.95%	P:oxidation reduction; P:proteolysis; F:2-alkenal reductase activity; F:cysteine-type endopeptidase activity
Mado-00-G1-K1-002_F10	453	armadillo beta-catenin repeat family protein	2.34477E-38	73.73%	F:binding; F:ligase activity

Mado-00-G1-K1-002_F11	387	4-hydroxyphenylpyruvate dioxygenase	3.15527E-35	96.15%	C:cytosol; F:4-hydroxyphenylpyruvate dioxygenase activity; C:mitochondrion; F:metal ion binding; P:oxidation reduction; P:vitamin E biosynthetic process; P:L-phenylalanine catabolic process; P:carotenoid biosynthetic process; P:plastoquinone biosynthetic process; P:tyrosine catabolic process; C:chloroplast
Mado-00-G1-K1-002_G02	246	aspartate aminotransferase	1.84656E-35	94.30%	P:leaf senescence; F:pyridoxal phosphate binding; F:L-aspartate:2-oxoglutarate aminotransferase activity; P:biosynthetic process; P:cellular amino acid metabolic process; C:membrane; C:plastid
Mado-00-G1-K1-002_G03	236	universal stress protein family protein	1.8765E-27	77.95%	P:response to stress
Mado-00-G1-K1-002_G07	283	unknown [Glycine max]	2.47562E-22	78.70%	C:respiratory chain complex I; P:photorespiration; C:mitochondrial membrane
Mado-00-G1-K1-002_G09	417	actin	6.23581E-71	99.00%	C:cytoplasm; P:auxin biosynthetic process; C:cytoskeleton; F:protein binding; F:ATP binding
Mado-00-G1-K1-002_G10	436	protease inhibitor seed storage lipid transfer protein family protein	1.32086E-41	69.60%	P:lipid transport; C:cytoplasmic membrane-bounded vesicle; F:lipid binding
Mado-00-G1-K1-002_G12	265	dna binding	5.32693E-6	61.67%	F:DNA binding; C:nucleus
Mado-00-G1-K1-002_H02	422	sodium calcium exchanger family-like protein	1.49178E-40	78.60%	C:plant-type vacuole; C:plasma membrane; P:transmembrane transport; C:vacuolar membrane; F:calcium ion binding; C:integral to membrane
Mado-00-G1-K1-002_H06	233	ubiquitin-conjugating enzyme e2	1.54032E-29	99.90%	F:ubiquitin-protein ligase activity; P:regulation of protein metabolic process; P:post-translational protein modification
Mado-00-G1-K1-002_H07	399	glycerol 3-phosphate permease	2.31009E-17	82.05%	F:RNA-directed DNA polymerase activity; P:RNA-dependent DNA replication; F:RNA binding; P:transmembrane transport; F:sugar:hydrogen symporter activity; P:carbohydrate transport; C:membrane; C:mitochondrion
Mado-00-G1-K1-002_H10	334	ankyrin protein	7.01249E-51	89.45%	C:cytoplasmic membrane-bounded vesicle; C:Golgi apparatus; C:nucleus; F:zinc ion binding; F:protein binding; C:plasma membrane; F:methyltransferase activity
Mado-00-G1-K1-002_H12	379	col domain class transcription factor	4.17106E-35	73.80%	F:kinase activity; F:zinc ion binding; C:intracellular
Mado-00-G1-K1-003B_A03	318	dcl protein	8.73544E-49	80.90%	C:mitochondrion; C:chloroplast
Mado-00-G1-K1-003B_A07	247	histone ubiquitination proteins group	6.58288E-25	97.70%	P:ubiquitin-dependent protein catabolic process; P:negative regulation of flower development; P:histone H2B ubiquitination; P:regulation of protein metabolic process; P:UV protection; P:leaf morphogenesis; P:vegetative to reproductive phase transition of meristem; F:ubiquitin-protein ligase activity
Mado-00-G1-K1-003B_A09	187	receptor serine threonine	2.03972E-21	87.05%	P:auxin biosynthetic process; F:receptor activity; P:protein amino acid phosphorylation; F:non-membrane spanning protein tyrosine kinase activity; F:ATP binding; F:protein serine/threonine kinase activity
Mado-00-G1-K1-003B_A12	181	clathrin adaptor complexes medium subunit family protein	1.55989E-26	99.50%	C:clathrin vesicle coat; F:protein binding; P:vesicle-mediated transport; P:intracellular protein transport; C:clathrin adaptor complex
Mado-00-G1-K1-003B_B03	301	receptor for activated protein kinase	2.43504E-35	89.00%	P:shoot development; F:myosin heavy chain kinase activity; F:receptor activity; P:root development; F:nucleotide binding
Mado-00-G1-K1-003B_B05	340	cullin-like 1 protein	1.4022E-51	94.40%	P:ubiquitin-dependent protein catabolic process; P:cell cycle; P:embryonic development ending in seed dormancy; P:jasmonic acid mediated signaling pathway; P:regulation of circadian rhythm; C:condensed nuclear chromosome; P:SCF complex assembly; C:cullin-RING ubiquitin ligase complex; F:ubiquitin protein ligase binding; P:response to auxin stimulus; C:spindle; C:phragmoplast
Mado-00-G1-K1-003B_B08	381	glycerol 3-phosphate permease	2.96343E-49	91.10%	F:RNA-directed DNA polymerase activity; P:RNA-dependent DNA replication; F:RNA binding; P:transmembrane transport; F:sugar:hydrogen symporter activity; P:carbohydrate transport; C:membrane
Mado-00-G1-K1-003B_B10b	206	glutamine synthetase	1.63604E-23	94.10%	C:cytoplasm; F:glutamate-ammonia ligase activity; P:nitrogen fixation; F:ATP binding; P:glutamine biosynthetic process
Mado-00-G1-K1-003B_C01	204	brassinosteroid-regulated protein bru1	9.31158E-11	86.00%	F:hydrolase activity, hydrolyzing O-glycosyl compounds; P:cellular glucan metabolic process; C:apoplast; C:cell wall; F:xyloglucan:xyloglucosyl transferase activity
Mado-00-G1-K1-003B_C03	293	fructose bisphosphate aldolase	7.39525E-40	87.40%	C:cytosol; F:copper ion binding; C:cell wall; C:nucleolus; P:pentose-phosphate shunt; P:response to salt stress; F:protein binding; P:glycolysis; C:mitochondrial envelope; F:fructose-bisphosphate aldolase activity; C:chloroplast; P:response to cadmium ion; C:plasma membrane; C:apoplast
Mado-00-G1-K1-003B_C05	419	protein	1.49806E-40	62.95%	F:transferase activity, transferring acyl groups other than amino-acyl groups
Mado-00-G1-K1-003B_C06	109	lhca1 chlorophyll binding	5.95462E-13	97.00%	C:plastoglobule; C:light-harvesting complex; P:photosynthesis, light harvesting in photosystem I; P:response to blue light; F:chlorophyll binding; P:response to far red light; P:response to red light; C:chloroplast thylakoid membrane
Mado-00-G1-K1-003B_C07	251	60s ribosomal protein	1.28325E-12	79.00%	C:ribosome; C:cytoplasmic membrane-bounded vesicle
Mado-00-G1-K1-003B_C10	336	set domain protein	4.13362E-19	74.35%	P:chromatin modification; F:histone-lysine N-methyltransferase activity; C:nucleus; F:zinc ion binding
Mado-00-G1-K1-003B_C10b	145	protein	2.05001E-21	96.60%	P:auxin biosynthetic process; C:nucleolus; C:chloroplast envelope; F:RNA helicase activity; F:ATP-dependent helicase activity; F:nucleic acid binding; F:ATP binding; C:membrane
Mado-00-G1-K1-003B_C11	230	molybdenum cofactor sulfurase family protein	1.00181E-28	86.85%	F:pyridoxal phosphate binding; F:Mo-molybdopterin cofactor sulfurase activity; F:molybdenum ion binding; C:chloroplast
Mado-00-G1-K1-003B_C12	419	gdp-d-mannose-3 -epimerase	1.44763E-43	93.80%	F:GDP-mannose 3,5-epimerase activity; P:cellular metabolic process; F:coenzyme binding
Mado-00-G1-K1-003B_D02	175	ap2 domain class transcription factor	3.74555E-20	81.85%	F:transcription factor activity; C:nucleus; P:regulation of transcription, DNA-dependent
Mado-00-G1-K1-003B_D03	341	26s proteasome atpase subunit	3.1529E-19	97.00%	C:cytoplasm; P:auxin biosynthetic process; F:peptidase activity; C:proteasome regulatory particle, base subcomplex; P:ubiquitin-dependent protein catabolic process; F:ATP binding; F:microtubule-severing ATPase activity; C:nucleus
Mado-00-G1-K1-003B_D05	325	conserved hypothetical protein [Ricinus communis]	6.86976E-46	85.35%	P:metal ion transport; C:endomembrane system; C:membrane; F:metal ion transmembrane transporter activity; P:transmembrane transport
Mado-00-G1-K1-003B_D06	167	phosphoenolpyruvate carboxylase	1.06574E-14	95.05%	C:cytoplasm; P:tricarboxylic acid cycle; F:phosphoenolpyruvate carboxylase activity; P:photosynthesis; P:carbon fixation
Mado-00-G1-K1-003B_D11	258	tubulin alpha	6.72345E-33	100.00%	F:structural constituent of cytoskeleton; P:microtubule-based movement; F:GTP binding; P:protein polymerization; C:cytosol; F:GTPase activity; C:microtubule
Mado-00-G1-K1-003B_D12	127	pre-mrna splicing factor prp19	1.10267E-11	86.90%	C:nucleolus; P:defense response to bacterium; P:response to cadmium ion; F:protein binding; P:protein ubiquitination; C:cell wall; C:CUL4 RING ubiquitin ligase complex; F:ubiquitin-protein ligase activity; C:chloroplast; F:nucleotide binding
Mado-00-G1-K1-003B_E05	201	protein	2.45854E-11	66.90%	C:cytoplasmic membrane-bounded vesicle; C:chloroplast
Mado-00-G1-K1-003B_E12	283	chlorophyll a b-binding	7.79022E-13	75.87%	C:chloroplast stromal thylakoid; C:plastoglobule; P:photosynthesis, light harvesting; P:response to blue light; F:chlorophyll binding; P:nonphotochemical quenching; C:PSII associated light-harvesting complex II; P:response to far red light; P:response to red light; C:photosystem II antenna complex
Mado-00-G1-K1-003B_F02	201	glutamate decarboxylase	9.93537E-29	95.45%	F:pyridoxal phosphate binding; F:calmodulin binding; P:glutamate metabolic process; F:glutamate decarboxylase activity
Mado-00-G1-K1-003B_F04	301	calcium-binding ef hand family protein	9.25316E-35	83.65%	F:calcium ion binding
Mado-00-G1-K1-003B_G01	149	s-adenosyl-l-homocysteine hydrolase	2.11481E-18	96.95%	F:adenosylhomocysteinase activity; P:one-carbon metabolic process; F:binding
Mado-00-G1-K1-003B_G04	454	at5g40510 mnf13_30	4.00886E-30	58.10%	C:mitochondrion; F:molecular_function; P:biological_process; C:cellular_component
Mado-00-G1-K1-003B_G05	459	neoxanthin cleavage enzyme-like protein	1.6464E-76	89.45%	C:plastoglobule; F:oxidoreductase activity, acting on single donors with incorporation of molecular oxygen, incorporation of two atoms of oxygen; F:protein binding
Mado-00-G1-K1-003B_G09	431	starch branching enzyme i	1.77979E-78	93.00%	F:hydrolase activity, hydrolyzing O-glycosyl compounds; F:cation binding; C:amyloplast; F:obs,4-alpha-glucan branching enzyme activity; P:starch biosynthetic process; C:chloroplast
Mado-00-G1-K1-003B_G11	183	protein	3.73799E-15	94.15%	F:asparagine-tRNA ligase activity; P:auxin biosynthetic process; P:asparaginyl-tRNA aminoacylation; F:nucleic acid binding; C:mitochondrion; P:aspartyl-tRNA aminoacylation; P:ovule development; F:ATP binding; F:aspartate-tRNA ligase activity; C:chloroplast
Mado-00-G1-K1-003B_H05	333	protein	4.56649E-34	76.95%	C:plasma membrane
Mado-00-G1-K1-003B_H06	292	rad23-like protein	3.4512E-21	94.90%	F:proteasome binding; P:response to cold; P:proteasomal ubiquitin-dependent protein catabolic process; F:damaged DNA binding; F:ubiquitin binding; P:nucleotide-excision repair; C:nucleus
Mado-00-G1-K1-003B_H08	331	dehydrin	4.6052E-18	68.05%	C:cytoplasm; P:response to water deprivation; F:actin binding; P:response to abscisic acid stimulus; P:cold acclimation; P:regulation of seed germination; C:membrane

	Mado-00-G1-K1-003B_H09	441	cytochrome b5	6.08164E-34	79.10%	F:heme binding; C:endoplasmic reticulum; C:membrane part
	Mado-00-G1-K1-003B_H11	256	s-adenosylmethionine decarboxylase	6.35909E-7	100.00%	P:spermine biosynthetic process; F:adenosylmethionine decarboxylase activity; P:spermidine biosynthetic process
	Mado-00-G1-K1-004_A01	272	proteasome chain protein	2.06555E-26	91.70%	C:cytoplasm; C:proteasome core complex; P:response to cadmium ion; C:plasma membrane; P:ubiquitin-dependent protein catabolic process; C:nucleus; C:apoplast; F:threonine-type endopeptidase activity
	Mado-00-G1-K1-004_A03	292	molybdenum cofactor sulfurase family protein	1.54917E-21	68.70%	F:catalytic activity; F:binding
	Mado-00-G1-K1-004_A05	509	s-adenosylmethionine synthetase	6.17211E-58	79.80%	C:cytoplasm; P:auxin biosynthetic process; F:methionine adenosyltransferase activity; P:one-carbon metabolic process; F:ATP binding; F:magnesium ion binding; P:response to salt stress
	Mado-00-G1-K1-004_A06	333	60s ribosomal protein	8.87082E-46	93.65%	C:ribosome; F:structural constituent of ribosome; P:translation; F:rRNA binding
	Mado-00-G1-K1-004_A08	538	cytosolic class i small heat shock protein 2b	3.98167E-51	66.90%	C:mitochondrion; P:response to stress
	Mado-00-G1-K1-004_A09	569	global transcription factor group	8.58167E-29	56.60%	F:acyltransferase activity; F:transferase activity; F:histone acetyltransferase activity; F:DNA binding
	Mado-00-G1-K1-004_A12	264	dna-directed rna polymerase	2.08023E-26	76.00%	F:DNA-directed RNA polymerase activity
	Mado-00-G1-K1-004_B02	305	histone 2	8.35778E-28	100.00%	P:defense response to bacterium; C:nucleosome; F:DNA binding; P:nucleosome assembly; F:protein binding; P:flower development; C:nucleus
	Mado-00-G1-K1-004_B04	223	galactose-1-phosphate uridyl transferase-like protein	1.19698E-13	84.57%	F:ribose-5-phosphate adenylyltransferase activity; F:UTP:galactose-1-phosphate uridylyltransferase activity; P:galactose metabolic process; F:zinc ion binding; F:UDP-glucose:hexose-1-phosphate uridylyltransferase activity; P:positive regulation of cellular response to phosphate starvation
	Mado-00-G1-K1-004_B07	237	udp-glucose:sterol glucosyltransferase	1.25438E-23	85.25%	P:carbohydrate metabolic process; P:lipid glycosylation; F:sterol 3-beta-glucosyltransferase activity; C:plasma membrane
	Mado-00-G1-K1-004_C02	160	kelch repeat-containing f-box family protein	7.17753E-19	82.50%	F:molecular_function; C:chloroplast; P:biological_process; C:cellular_component
	Mado-00-G1-K1-004_C07	539	26s proteasome regulatory subunit	6.08612E-84	86.05%	C:eukaryotic translation initiation factor 3 complex; P:translational initiation; F:translation initiation factor activity; C:proteasome complex; C:membrane; C:nucleus
	Mado-00-G1-K1-004_D01	213	protein	8.33818E-12	96.55%	F:molecular_function; P:biological_process
	Mado-00-G1-K1-004_D03	354	zip transporter	8.93896E-46	92.05%	P:metal ion transport; C:endomembrane system; C:membrane; C:cytoplasmic membrane-bounded vesicle; F:metal ion transmembrane transporter activity; P:transmembrane transport
	Mado-00-G1-K1-004_D06	440	aldo keto reductase family protein	1.14151E-69	91.40%	F:steroid dehydrogenase activity; C:cytosol; P:response to water deprivation; P:response to salt stress; P:oxidation reduction; C:nucleus; P:response to cold; F:aldehyde reductase activity
	Mado-00-G1-K1-004_D10	213	alliinase family protein	1.20124E-18	76.40%	F:pyridoxal phosphate binding; F:alliin lyase activity
	Mado-00-G1-K1-004_E02	481	lipid transfer protein precursor	2.38775E-59	89.75%	F:lipid binding; P:lipid transport
	Mado-00-G1-K1-004_E03	302	glutaredoxin c4	2.07578E-10	93.80%	C:endomembrane system; P:cell redox homeostasis; F:electron carrier activity; F:protein disulfide oxidoreductase activity; F:arsenate reductase (glutaredoxin) activity
	Mado-00-G1-K1-004_E04	315	peroxidase	1.89195E-43	93.10%	F:heme binding; P:oxidation reduction; F:peroxidase activity; F:protein binding; P:response to oxidative stress
	Mado-00-G1-K1-004_E05	526	tryptophan synthase alpha chain	2.99191E-52	85.55%	P:callose deposition in cell wall during defense response; C:peroxisome; F:calcium ion binding; P:defense response to bacterium; F:tryptophan synthase activity; F:protein binding; C:chloroplast stroma; P:tryptophan biosynthetic process; C:vacuole; P:defense response to fungus
	Mado-00-G1-K1-004_E06	379	protein	1.20518E-58	88.35%	C:mitochondrion; C:membrane
	Mado-00-G1-K1-004_E08	390	temperature-induced lipocalin	2.58266E-21	82.70%	P:transport; F:lipid binding; F:transporter activity
	Mado-00-G1-K1-004_E12	382	protein	7.6062E-21	63.25%	P:cell death; C:integral to membrane
	Mado-00-G1-K1-004_F02	312	protein	1.56432E-21	68.40%	C:plasma membrane
	Mado-00-G1-K1-004_F05	338	alanine acetyl transferase	7.75959E-34	74.80%	P:response to abscisic acid stimulus; P:metabolic process; F:N-acetyltransferase activity
	Mado-00-G1-K1-004_F06	290	at3g46970 f13i12_20	1.30709E-44	93.05%	P:response to water deprivation; F:phosphorylase activity; P:carbohydrate metabolic process; F:pyridoxal phosphate binding; C:chloroplast; C:cytosol
	Mado-00-G1-K1-004_F07	186	atp synthase beta chain	1.23459E-26	100.00%	P:plasma membrane ATP synthesis coupled proton transport; P:auxin biosynthetic process; F:hydrogen-exporting ATPase activity, phosphorylative mechanism; F:hydrogen ion transporting ATP synthase activity, rotational mechanism; C:mitochondrial proton-transporting ATP synthase complex, catalytic core F(1); F:ATP binding; F:proton-transporting ATPase activity, rotational mechanism
	Mado-00-G1-K1-004_F08	219	low-temperature-induced 65 kd protein	1.67977E-7	57.20%	P:abscisic acid mediated signaling pathway; P:response to water deprivation; P:response to salt stress; P:response to cold
	Mado-00-G1-K1-004_G02	384	at3g10250 f14p13_15	1.67596E-44	79.10%	F:molecular_function; P:biological_process; C:cellular_component
	Mado-00-G1-K1-004_G06	401	actin interacting protein	6.72147E-53	80.10%	F:FAD binding; P:oxidation reduction; C:mitochondrion; F:electron carrier activity; F:(R)-2-hydroxyglutarate dehydrogenase activity; F:D-lactate dehydrogenase (cytochrome) activity
	Mado-00-G1-K1-004_G07	218	seed maturation-like protein	2.49635E-16	81.86%	C:chloroplast
	Mado-00-G1-K1-004_G08	288	metallothionein-like protein	5.72257E-16	81.45%	F:metal ion binding
	Mado-00-G1-K1-004_G12	403	ribulose bisphosphate carboxylase	1.63729E-39	89.35%	P:reductive pentose-phosphate cycle; C:cytosolic ribosome; F:ribulose-bisphosphate carboxylase activity; F:monooxygenase activity; P:photorespiration; F:protein binding; C:cell wall; P:response to blue light; P:oxidation reduction; C:thylakoid; C:membrane; C:chloroplast ribulose bisphosphate carboxylase complex; C:apoplast; P:response to red light; P:response to far red light
	Mado-00-G1-K1-004_H01	352	heat shock protein binding protein	8.71202E-33	80.40%	P:protein folding; P:response to stress; F:heat shock protein binding
	Mado-00-G1-K1-004_H02	344	unnamed protein product [Vitis vinifera]	8.02714E-10	65.00%	F:binding
	Mado-00-G1-K1-004_H03	306	protein	3.2821E-16	71.95%	F:molecular_function; C:chloroplast; P:biological_process
	Mado-00-G1-K1-004_H06	258	membrane steroid-binding protein 1	2.48038E-19	74.35%	C:plasma membrane; P:negative regulation of cell growth; F:heme binding; F:steroid binding; C:nucleus; C:endoplasmic reticulum; C:chloroplast thylakoid membrane; P:electron transport chain; F:2-alkenal reductase activity
	Mado-00-G1-K1-004_H08	252	short-chain dehydrogenase reductase family protein	1.49542E-25	79.60%	P:response to cadmium ion; P:oxidation reduction; F:tropine dehydrogenase activity; F:protein binding
G2-K2	Mado-00-G2-K2_Contig1	773	trehalose 6-phosphate synthase	8.68585E-62	86.60%	P:trehalose biosynthetic process; F:transferase activity; F:alpha,alpha-trehalose-phosphate synthase (UDP-forming) activity; F:hydrolase activity; P:metabolic process; F:catalytic activity; F:trehalose-phosphatase activity; F:transferase activity, transferring glycosyl groups
	Mado-00-G2-K2_Contig2	290	dehydrin-like protein	1.25606E-20	67.75%	P:response to water; P:response to stress
	Mado-00-G2-K2_Contig3	322	protein	9.56853E-21	80.75%	C:plant-type cell wall; P:defense response; P:response to brassinosteroid stimulus; P:response to gibberellin stimulus; P:response to abscisic acid stimulus; F:molecular_function; C:extracellular region; F:protein binding; C:cell wall; P:unidimensional cell growth
	Mado-00-G2-K2_Contig4	407	yth domain-containing	1.78444E-27	77.00%	F:molecular_function; C:cytoplasm; P:biological_process; F:protein binding; C:cellular_component; C:nucleus
	Mado-00-G2-K2_Contig5	390	temperature-induced lipocalin	3.80234E-22	84.15%	F:lipid binding; F:binding; F:transporter activity; P:transport
	Mado-00-G2-K2_Contig6	336	dehydrin	2.63982E-23	74.70%	P:response to water; P:response to stress
	Mado-00-G2-K2_Contig8	365	calmodulin-binding protein (CAMTA1)	1.93908E-50	86.10%	F:transcription activator activity; P:regulation of transcription; P:biological_process; F:calmodulin binding; F:transcription regulator activity; C:cellular_component; P:response to freezing; C:nucleus
	Mado-00-G2-K2_Contig10	233	hypothetical protein [Vitis vinifera]	0.0341242	50.00%	
	Mado-00-G2-K2_Contig12	458	zinc finger	3.01088E-35	68.10%	C:endomembrane system; P:lipid metabolic process; C:mitochondrion; C:cytoplasmic membrane-bounded vesicle; F:hydrolase activity, acting on ester bonds; F:carboxylesterase activity
	Mado-00-G2-K2_Contig13	436	protein	3.56043E-28	48.05%	P:biological_process
	Mado-00-G2-K2_Contig14	432	sucrose synthase	1.2659E-41	88.80%	F:transferase activity; C:plant-type cell wall; F:sucrose synthase activity; P:biosynthetic process; P:sucrose biosynthetic process; C:membrane; P:sucrose metabolic process; P:response to hypoxia; F:transferase activity, transferring glycosyl groups; F:UDP-glycosyltransferase activity; F:molecular_function; C:cellular_component; P:suspensor development
	Mado-00-G2-K2_Contig15	387	constans-like protein	1.02771E-59	70.15%	F:zinc ion binding; C:intracellular

Mado-00-G2-K2_Contig16	303	peptide transporter-like protein	2.92199E-38	78.35%	P:oligopeptide transport; C:integral to membrane; C:membrane; F:transporter activity; P:transport
Mado-00-G2-K2_Contig17	297	protein	1.08339E-32	70.22%	F:molecular_function; P:biological_process; C:cellular_component
Mado-00-G2-K2_Contig18	291	xyloglucan galactosyltransferase	9.2385E-32	75.05%	C:mitochondrion; C:cell wall; C:cytoplasmic membrane-bounded vesicle; F:transferase activity; F:catalytic activity; C:membrane; P:biological_process; F:transferase activity, transferring glycosyl groups
Mado-00-G2-K2_Contig19	282	protein	2.38044E-27	72.84%	C:plastid
Mado-00-G2-K2_Contig20	244	protein (FUS)	1.50042E-26	89.05%	C:plastid; F:RNA binding; F:nucleic acid binding; P:RNA splicing; F:nucleotide binding
Mado-00-G2-K2_Contig21	234	rna-binding region rnp-1 and splicing factor pwi family member protein	1.92215E-13	61.25%	C:plastid; F:nucleic acid binding; F:nucleotide binding; P:mRNA processing
Mado-00-G2-K2_Contig22	210	galactinol synthase	6.2821E-33	92.65%	F:transferase activity; F:inositol 3-alpha-galactosyltransferase activity; F:transferase activity, transferring glycosyl groups
Mado-00-G2-K2_Contig23	170	at3g46970 f13i12_20	1.46054E-21	90.10%	F:transferase activity; F:pyridoxal phosphate binding; F:phosphorylase activity; F:transferase activity, transferring glycosyl groups; C:cytoplasm; P:carbohydrate metabolic process; P:response to water deprivation; C:cytosol; C:chloroplast
Mado-00-G2-K2_Contig24	608	PREDICTED: hypothetical protein [Vitis vinifera]	9.76292E-45	67.00%	F:molecular_function; P:biological_process; C:cellular_component
Mado-00-G2-K2_Contig25	543	senescence-associated protein	7.4524E-50	78.65%	F:molecular_function; C:cytoplasmic membrane-bounded vesicle; C:integral to membrane; C:membrane; P:aging
Mado-00-G2-K2_Contig26	472	l-asparaginase l-asparagine amidohydrolase	1.4176E-16	87.00%	P:glycoprotein catabolic process; F:beta-aspartyl-peptidase activity; F:hydrolase activity; F:asparaginase activity; F:peptidase activity; C:cellular_component
Mado-00-G2-K2_Contig27	468	cc-nbs-lrr resistance protein	0.00402227	64.50%	F:ATP binding; P:apoptosis; P:defense response; F:nucleotide binding; F:protein binding
Mado-00-G2-K2_Contig28	407	protein	1.95471E-58	83.35%	C:heterotrimeric G-protein complex; F:molecular_function; P:biological_process; C:CUL4 RING ubiquitin ligase complex
Mado-00-G2-K2_Contig29	393	ribulose bisphosphate carboxylase	3.18718E-40	89.35%	C:apoplast; P:response to far red light; C:chloroplast; C:plastid; F:ribulose-bisphosphate carboxylase activity; C:membrane; C:cell wall; C:thylakoid; C:cytosolic ribosome; P:photorespiration; C:chloroplast ribulose bisphosphate carboxylase complex; F:lyase activity; P:response to red light; C:chloroplast stroma; P:photosynthesis; P:response to blue light; P:carbon fixation; P:oxidation reduction; P:reductive pentose-phosphate cycle; F:monooxygenase activity; F:protein binding; F:oxidoreductase activity
Mado-00-G2-K2_Contig30	373	conserved protein	1.43702E-8	64.88%	
Mado-00-G2-K2_Contig31	369	atp-binding cassette	1.37809E-51	84.35%	P:negative regulation of defense response; P:drug transmembrane transport; C:chloroplast; P:defense response to bacterium; F:hydrolase activity; P:defense response to fungus, incompatible interaction; F:cadmium ion transmembrane transporter activity; F:ATP binding; P:systemic acquired resistance; C:membrane; P:callose deposition in cell wall during defense response; C:mitochondrion; P:auxin biosynthetic process; F:nucleotide binding; F:ATPase activity; F:nucleoside-triphosphatase activity; F:ATPase activity, coupled to transmembrane movement of substances; P:indole glucosinolate catabolic process; P:cadmium ion transport; C:plasma membrane; F:phosphonate transmembrane-transporting ATPase activity
Mado-00-G2-K2_Contig32	361	heat shock protein	4.09712E-5	72.33%	F:ATP binding; P:response to stress; P:protein metabolic process; F:nucleotide binding; F:nucleoside-triphosphatase activity; P:auxin biosynthetic process; F:protein binding
Mado-00-G2-K2_Contig33	347	tif3b1 (translation initiation factor 3b1) nucleic acid binding translation initiation factor	8.88948E-59	93.15%	C:eukaryotic translation initiation factor 3 complex; F:DNA binding; C:nucleus; P:translational initiation; F:nucleic acid binding; F:nucleotide binding; C:cytoplasm; P:translation; F:RNA binding; F:protein binding; F:translation initiation factor activity; C:cytosol
Mado-00-G2-K2_Contig34	338	ribulose bisphosphate carboxylase	1.49551E-26	92.05%	P:photorespiration; P:photosynthesis; P:carbon fixation; P:oxidation reduction; F:lyase activity; F:oxidoreductase activity; P:reductive pentose-phosphate cycle; C:chloroplast ribulose bisphosphate carboxylase complex; F:ribulose-bisphosphate carboxylase activity; F:monooxygenase activity; C:plastid; C:chloroplast
Mado-00-G2-K2_Contig35	308	gras family transcription factor	8.47038E-38	84.75%	P:transcription; P:regulation of transcription
Mado-00-G2-K2_Contig36	295	protein	2.34625E-27	76.70%	F:molecular_function; P:biological_process; C:cellular_component
Mado-00-G2-K2_Contig37	285	serine decarboxylase	2.61029E-42	89.50%	F:pyridoxal phosphate binding; P:cellular amino acid metabolic process; P:embryonic development ending in seed dormancy; F:lyase activity; F:carboxy-lyase activity; F:catalytic activity; C:cellular_component; F:histidine decarboxylase activity; P:carboxylic acid metabolic process
Mado-00-G2-K2_Contig38	279	fibrillin precursor-like protein	2.09325E-12	88.95%	C:chloroplast; C:plastid; C:nucleus; C:chloroplast thylakoid membrane; F:molecular_function; P:response to abscisic acid stimulus; F:structural molecule activity; C:membrane; C:thylakoid lumen; C:plastoglobule; C:thylakoid; P:response to cold; C:stromule; P:biological_process; C:chloroplast stroma; P:response to stress; P:photoinhibition; F:protein binding
Mado-00-G2-K2_Contig39	267	protein	3.58838E-44	97.15%	F:transferase activity; F:protein serine/threonine kinase activity; F:protein kinase activity; P:protein amino acid phosphorylation; F:non-membrane spanning protein tyrosine kinase activity; F:ATP binding; F:kinase activity; F:protein binding; C:plasma membrane
Mado-00-G2-K2_Contig40	263	cysteine protease	2.00143E-10	81.65%	F:cysteine-type endopeptidase activity; F:hydrolase activity; P:aging; F:cysteine-type peptidase activity; F:peptidase activity; P:proteolysis; F:protein binding; C:endomembrane system; P:response to ethylene stimulus
Mado-00-G2-K2_Contig41	261	60s ribosomal protein l15	1.67867E-41	97.75%	C:plastid; F:structural constituent of ribosome; C:mitochondrion; P:translation; C:ribonucleoprotein complex; C:ribosome; C:intracellular
Mado-00-G2-K2_Contig42	261	zinc finger (c3hc4-type ring finger) family protein	8.08809E-28	74.75%	F:metal ion binding; F:zinc ion binding; F:protein binding
Mado-00-G2-K2_Contig43	257	mutt nudix	1.43772E-24	79.79%	F:metal ion binding; C:chloroplast; F:hydrolase activity; P:biological_process; F:NAD+ diphosphatase activity
Mado-00-G2-K2_Contig44	232	ap2 domain-containing transcription factor	1.3295E-30	98.85%	P:seed development; P:specification of floral organ identity; P:sexual reproduction; F:2-alkenal reductase activity; F:transcription factor activity; F:DNA binding; C:nucleus; P:oxidation reduction; P:cell differentiation; F:oxidoreductase activity; P:regulation of transcription, DNA-dependent; P:flower development; P:meristem maintenance; P:transcription; C:plastid; P:regulation of transcription
Mado-00-G2-K2_Contig45	219	purple acid phosphatase	4.33515E-29	89.00%	F:metal ion binding; C:cytoplasmic membrane-bounded vesicle; F:hydrolase activity; F:acid phosphatase activity; P:biological_process; F:protein serine/threonine phosphatase activity
Mado-00-G2-K2_Contig46	219	low-temperature-induced 65 kd protein	6.09143E-7	54.68%	P:abscisic acid mediated signaling pathway; F:molecular_function; P:response to water deprivation; P:response to abscisic acid stimulus; P:response to salt stress; P:response to cold; C:cellular_component
Mado-00-G2-K2_Contig47	212	integral membrane hrf1 family protein	3.33879E-26	87.95%	F:molecular_function; C:integral to membrane; C:membrane; P:biological_process
Mado-00-G2-K2_Contig49	209	actin-related protein 6	5.16821E-27	84.40%	F:structural constituent of cytoskeleton; C:chromatin remodeling complex; P:chromatin remodeling; P:actin filament-based process; P:cell proliferation; P:negative regulation of flower development; F:protein binding; C:nucleus
Mado-00-G2-K2_Contig50	191	beta-amylase	1.81176E-27	89.35%	P:carbohydrate metabolic process; P:metabolic process; F:hydrolase activity; F:catalytic activity; F:hydrolase activity, acting on glycosyl bonds; F:cation binding; P:polysaccharide catabolic process; F:beta-amylase activity
Mado-00-G2-K2_Contig51	191	PREDICTED: hypothetical protein [Vitis vinifera]	0.0154445	53.00%	P:regulation of transcription; F:DNA binding; C:nucleus
Mado-00-G2-K2_Contig52	162	white-brown-complex abc transporter family	1.12545E-21	85.75%	F:ATPase activity, coupled to transmembrane movement of substances; P:cutin transport; P:auxin biosynthetic process; C:membrane; F:hydrolase activity; F:phosphonate transmembrane-transporting ATPase activity; F:nucleotide binding; F:nucleoside-triphosphatase activity; F:ATP binding; C:external side of plasma membrane; F:ATPase activity; P:fatty acid transport; C:plasma membrane; F:fatty acid transporter activity

Mado-00-G2-K2_Contig53	139	aldose reductase	1.03695E-19	81.05%	F:steroid dehydrogenase activity; C:nucleus; F:aldo-keto reductase activity; F:aldehyde reductase activity; P:oxidation reduction; P:response to water deprivation; F:oxidoreductase activity; P:response to cold; P:response to salt stress; C:cytosol; P:response to cadmium ion
Mado-00-G2-K2_Contig54	132	hypothetical protein RCOM_0555710 [Ricinus communis]	0.0197692	63.00%	F:zinc ion binding; C:intracellular
Mado-00-G2-K2_Contig55	118	late embryogenesis	0.00404492	66.17%	
Mado-00-G2-K2-001B_A04	400	protein	1.28344E-41	77.90%	F:binding; P:biological_process; C:cellular_component
Mado-00-G2-K2-001B_A06	216	at1g78070 f28k19_28	1.35274E-11	67.60%	F:molecular_function; P:biological_process; C:CUL4 RING ubiquitin ligase complex
Mado-00-G2-K2-001B_B04	272	waxy 2	2.7378E-44	92.95%	F:transferase activity; P:biosynthetic process; C:amyloplast; F:transferase activity, transferring glycosyl groups; P:glycogen biosynthetic process; P:starch biosynthetic process; F:starch synthase activity; P:glucan biosynthetic process; C:plastid; C:chloroplast
Mado-00-G2-K2-001B_B05	252	starch branching enzyme i	4.81396E-12	100.00%	F:hydrolase activity, hydrolyzing O-glycosyl compounds; P:carbohydrate metabolic process; F:catalytic activity; F:cation binding
Mado-00-G2-K2-001B_B09	771	aspartic proteinase	1.02365E-70	84.40%	C:cytoplasmic membrane-bounded vesicle; F:endopeptidase activity; C:vacuole; P:lipid metabolic process; F:hydrolase activity; F:aspartic-type endopeptidase activity; F:peptidase activity; P:response to salt stress; P:proteolysis
Mado-00-G2-K2-001B_C02	334	calcium ion binding	3.33664E-34	85.70%	C:plant-type vacuole; C:vacuolar membrane; F:calcium ion binding; C:vacuole; C:integral to membrane; C:membrane; P:transmembrane transport; C:plasma membrane
Mado-00-G2-K2-001B_C04	301	h(\+)-transporting atpase plant fungi plasma membrane	3.03492E-35	98.40%	F:ATPase activity, uncoupled; P:auxin biosynthetic process; C:integral to membrane; C:membrane; P:cation transport; F:ATPase activity, coupled to transmembrane movement of ions, phosphorylative mechanism; F:hydrolase activity; F:nucleotide binding; F:ATP binding; F:ATPase activity; P:ATP biosynthetic process; F:catalytic activity; F:hydrogen-exporting ATPase activity, phosphorylative mechanism; P:metabolic process; P:proton transport; C:plasma membrane; F:hydrolase activity, acting on acid anhydrides, catalyzing transmembrane movement of substances
Mado-00-G2-K2-001B_C08	269	elongation factor 1-gamma	1.72494E-38	90.35%	P:translational elongation; C:mitochondrion; F:translation elongation factor activity; P:translation; F:transferase activity; C:eukaryotic translation elongation factor 1 complex
Mado-00-G2-K2-001B_C09	294	bzip protein	6.81072E-35	79.35%	F:molecular_function; C:plasma membrane
Mado-00-G2-K2-001B_C12	293	kinase family protein	3.16053E-48	95.55%	F:kinase activity; F:protein kinase activity; F:ATP binding; F:nucleotide binding; P:protein amino acid phosphorylation; P:auxin biosynthetic process; C:cellular_component; F:protein serine/threonine kinase activity
Mado-00-G2-K2-001B_D03	290	selenium-binding protein	7.06649E-48	92.15%	F:selenium binding; P:polar nucleus fusion; C:cellular_component
Mado-00-G2-K2-001B_D06	138	atp-binding cassette	1.45031E-13	90.90%	P:auxin biosynthetic process; C:membrane; F:hydrolase activity; F:phosphonate transmembrane-transporting ATPase activity; F:nucleotide binding; F:nucleoside-triphosphatase activity; F:ATP binding; F:ATPase activity; F:heme-transporting ATPase activity
Mado-00-G2-K2-001B_D09	348	cysteine protease	2.18112E-57	91.05%	F:cysteine-type endopeptidase activity; F:hydrolase activity; P:aging; F:cysteine-type peptidase activity; F:peptidase activity; P:proteolysis; F:protein binding; C:endomembrane system; P:response to ethylene stimulus
Mado-00-G2-K2-001B_E01	507	magnesium transporter	7.66822E-53	69.35%	C:membrane; F:magnesium ion transmembrane transporter activity; P:transmembrane transport; P:metal ion transport; C:plasma membrane; F:metal ion transmembrane transporter activity
Mado-00-G2-K2-001B_E02	298	at1g49820 f10f5_1	6.12338E-44	89.95%	F:kinase activity; F:S-methyl-5-thioribose kinase activity; F:transferase activity; P:L-methionine salvage from methylthioadenosine; P:methionine biosynthetic process; C:cellular_component
Mado-00-G2-K2-001B_E06	267	dna damage-binding	3.35862E-42	82.45%	P:response to UV; P:protein polyubiquitination; C:protein complex; C:nucleus; F:DNA binding; F:nucleic acid binding; P:nucleotide-excision repair, DNA damage removal; P:pyrimidine dimer repair; P:response to UV-B; F:ubiquitin-protein ligase activity; F:damaged DNA binding; F:nucleotide binding; P:nucleotide-excision repair; P:protein autoubiquitination; C:nucleoplasm; F:zinc ion binding; P:DNA repair; F:protein binding; C:CUL4 RING ubiquitin ligase complex
Mado-00-G2-K2-001B_E10	215	emsy n terminus domain-containing protein ent domain-containing protein	1.18354E-23	69.70%	P:oxidation reduction; F:molecular_function; F:oxidoreductase activity; P:metabolic process; P:biological_process; F:monooxygenase activity; C:cellular_component
Mado-00-G2-K2-001B_E11	167	kelch repeat-containing protein	8.60982E-14	96.10%	F:molecular_function; P:biological_process; F:protein binding; C:cellular_component
Mado-00-G2-K2-001B_F03	225	root hair defective 3 gtp-binding family protein	1.58119E-31	89.60%	P:cell tip growth; C:integral to membrane; C:membrane; F:hydrolase activity; F:nucleotide binding; C:cytoplasm; F:GTP binding; P:ER to Golgi vesicle-mediated transport; P:root epidermal cell differentiation; C:cellular_component; P:plant-type cell wall biogenesis; C:endoplasmic reticulum; P:actin cytoskeleton organization
Mado-00-G2-K2-001B_F04	263	lem3 (ligand-effect modulator 3) family protein	3.27612E-37	88.35%	C:plastid; P:phospholipid transport; C:chloroplast thylakoid lumen; P:cell division; C:membrane; C:Golgi apparatus; P:biological_process
Mado-00-G2-K2-001B_F11	352	inducer of cbf expression 1	3.74057E-25	60.10%	C:chloroplast; P:regulation of transcription; P:guard mother cell differentiation; F:transcription regulator activity; F:DNA binding; F:transcription factor activity; P:response to freezing; C:nucleus
Mado-00-G2-K2-001B_G01	396	protein	1.9744E-66	96.00%	F:ligase activity; P:regulation of protein metabolic process; P:post-translational protein modification; F:small conjugating protein ligase activity
Mado-00-G2-K2-001B_G04	275	sodium calcium exchanger family-like protein	6.30384E-33	86.75%	C:plant-type vacuole; C:vacuolar membrane; F:calcium ion binding; C:vacuole; C:integral to membrane; C:membrane; P:transmembrane transport; C:plasma membrane
Mado-00-G2-K2-001B_G08	398	26s non-atpase regulatory subunit	6.15187E-60	97.60%	C:proteasome complex; P:protein catabolic process; P:response to salt stress; P:ubiquitin-dependent protein catabolic process; C:nucleus; C:proteasome regulatory particle, lid subcomplex
Mado-00-G2-K2-001B_G09	186	eukaryotic translation initiation factor 2 beta subunit	4.96147E-17	82.60%	F:metal ion binding; P:translational initiation; P:translation; F:translation initiation factor activity
Mado-00-G2-K2-001B_H05	273	monodehydroascorbate reductase	6.28924E-41	88.55%	C:peroxisome; F:monodehydroascorbate reductase (NADH) activity; F:FAD binding; C:cytoplasm; C:apoplast; P:oxidation reduction; F:oxidoreductase activity; P:hydrogen peroxide catabolic process; C:peroxisomal matrix; C:plasma membrane; C:chloroplast
Mado-00-G2-K2-001B_H07	526	short-chain dehydrogenase reductase family protein	4.24875E-44	84.20%	P:oxidation reduction; F:binding; F:oxidoreductase activity; P:metabolic process; F:prostaglandin-E2 9-reductase activity; F:catalytic activity; C:cellular_component
Mado-00-G2-K2-001B_H09	215	ribosomal rna	3.12906E-8	82.00%	C:plastid; F:nucleic acid binding; F:methyltransferase activity; P:rRNA processing; F:transferase activity; P:rRNA methylation; P:methylation; C:nucleus
Mado-00-G2-K2-002C_A04	334	aspartate aminotransferase	1.44063E-53	96.10%	P:biosynthetic process; C:apoplast; C:chloroplast; C:plastid; F:transaminase activity; C:mitochondrial matrix; F:transferase activity, transferring nitrogenous groups; P:response to cadmium ion; P:cellular amino acid metabolic process; P:response to cold; P:2-oxoglutarate metabolic process; C:stromule; C:mitochondrion; F:L-aspartate:2-oxoglutarate aminotransferase activity; F:catalytic activity; C:chloroplast stroma; P:glutamate metabolic process; P:aspartate metabolic process; F:transferase activity, F:pyridoxal phosphate binding
Mado-00-G2-K2-002C_A09	286	sugar transporter family expressed	1.2606E-20	76.55%	F:sugar:hydrogen symporter activity; F:transporter activity; C:integral to membrane; C:membrane; F:2-alkenal reductase activity; P:carbohydrate transport; P:transport; F:substrate-specific transmembrane transporter activity; P:oxidation reduction; F:oxidoreductase activity; C:chloroplast envelope; F:carbohydrate transmembrane transporter activity; P:transmembrane transport; C:plastid; C:chloroplast
Mado-00-G2-K2-002C_A12	313	protein	1.64288E-36	85.25%	F:metal ion binding; P:oxidation reduction; F:oxidoreductase activity, acting on single donors with incorporation of molecular oxygen, incorporation of two atoms of oxygen; P:L-methionine salvage from methylthioadenosine; C:cellular_component; F:acireductone dioxygenase [iron(II)-requiring] activity; F:dioxygenase activity
Mado-00-G2-K2-002C_B01	340	myo-inisitol oxygenase	6.85644E-56	92.60%	P:oxidation reduction; P:syncytium formation; F:oxidoreductase activity; C:cytoplasm; P:inositol catabolic process; F:iron ion binding; F:inositol oxygenase activity

Mado-00-G2-K2-002C_B02	206	alpha-glucan water chloroplast	1.19543E-23	86.70%	C:chloroplast; C:plastid; P:carbohydrate metabolic process; P:response to symbiotic fungus; F:ATP binding; F:metal ion binding; F:kinase activity; F:protein histidine kinase activity; P:starch catabolic process; C:mitochondrion; F:catalytic activity; F:nucleotide binding; C:chloroplast stroma; F:alpha-glucan, water dikinase activity; P:phosphorylation; P:cold acclimation; F:protein binding; F:transferase activity
Mado-00-G2-K2-002C_B03	222	unnamed protein product [Vitis vinifera]	3.55913E-7	68.00%	P:apoptosis; F:protein binding
Mado-00-G2-K2-002C_B07	288	cysteine protease	1.80117E-43	89.30%	C:endomembrane system; P:proteolysis; C:cytoplasmic membrane-bounded vesicle; F:hydrolase activity; F:cysteine-type peptidase activity; F:peptidase activity; F:cysteine-type endopeptidase activity
Mado-00-G2-K2-002C_C01	310	protein	6.65573E-51	95.70%	P:detection of bacterium; F:ADP-ribose pyrophosphohydrolase activity; F:lyase activity; P:response to vitamin B1; P:thiamin biosynthetic process; F:iron-sulfur cluster binding; F:catalytic activity; C:chloroplast stroma; C:plastid; C:chloroplast
Mado-00-G2-K2-002C_C05	264	aldo keto	5.3936E-32	94.15%	P:oxidation reduction; F:pyridoxine 4-dehydrogenase activity; F:oxidoreductase activity; P:auxin mediated signaling pathway
Mado-00-G2-K2-002C_C06	179	translationally controlled tumor protein	9.37604E-21	87.80%	C:cytoplasm
Mado-00-G2-K2-002C_C08	318	serine palmitoyltransferase	5.40337E-48	86.35%	F:transferase activity; F:pyridoxal phosphate binding; P:photomorphogenesis; P:biosynthetic process; C:membrane; P:sphingosine biosynthetic process; P:pollen development; F:catalytic activity; F:protein binding; P:sphingolipid biosynthetic process; F:acyltransferase activity; C:endoplasmic reticulum; P:metabolic process; F:transferase activity, transferring nitrogenous groups; F:serine C-palmitoyltransferase activity
Mado-00-G2-K2-002C_C12	161	histone h3	6.19519E-20	96.00%	C:chromosome; C:nucleosome; P:nucleosome assembly; F:DNA binding; C:nucleus
Mado-00-G2-K2-002C_D02	190	carrier protein precursor-like	2.89251E-25	95.70%	C:mitochondrion; F:binding; C:integral to membrane; C:membrane; P:transmembrane transport; C:mitochondrial inner membrane; F:transporter activity; P:transport
Mado-00-G2-K2-002C_D03	245	xanthine uracil permease family expressed	5.9483E-23	71.10%	F:transmembrane transporter activity; C:membrane; P:transmembrane transport; F:transporter activity; P:transport
Mado-00-G2-K2-002C_D04	309	coat protein	8.41952E-46	84.30%	C:viral capsid; F:structural molecule activity
Mado-00-G2-K2-002C_D05	311	vacuolar-processing enzyme	2.00864E-39	84.05%	P:proteolysis; C:lytic vacuole; P:vacuolar protein processing; F:hydrolase activity; F:cysteine-type peptidase activity; F:peptidase activity; F:cysteine-type endopeptidase activity
Mado-00-G2-K2-002C_D06	198	ccch-type zinc finger protein	1.67915E-25	77.60%	F:transcription factor activity; F:DNA binding; F:nucleic acid binding; C:cytoplasm; F:zinc ion binding; F:RNA binding; C:cellular_component; F:metal ion binding; P:regulation of transcription
Mado-00-G2-K2-002C_D07	307	protein kinase	3.91643E-51	98.70%	F:transferase activity; P:auxin biosynthetic process; F:protein serine/threonine kinase activity; F:protein kinase activity; P:protein amino acid phosphorylation; F:nucleotide binding; F:ATP binding; F:kinase activity; F:MAP kinase kinase kinase activity
Mado-00-G2-K2-002C_D11	423	lustrin a-like	4.88351E-33	85.45%	P:biological_process; C:cellular_component
Mado-00-G2-K2-002C_E01	525	dormancy-associated mads-box transcription factor	8.01451E-36	84.05%	P:regulation of transcription, DNA-dependent; P:transcription; F:sequence-specific DNA binding; P:regulation of transcription; F:DNA binding; F:transcription factor activity; C:nucleus
Mado-00-G2-K2-002C_E02	292	protein	1.68365E-33	82.05%	P:oxidation reduction; F:oxidoreductase activity; F:aromatase activity
Mado-00-G2-K2-002C_E03	335	tir-nbs-lrr resistance protein	1.45743E-13	70.40%	P:defense response; F:transmembrane receptor activity; F:sugar binding; P:apoptosis; F:ATP binding; C:intrinsic to membrane; P:recognition of pollen; P:signal transduction; F:protein binding; P:innate immune response
Mado-00-G2-K2-002C_E10	247	heat shock protein 101	2.15411E-33	91.60%	F:ATP binding; P:response to stress; P:protein metabolic process; F:nucleotide binding; F:nucleoside-triphosphatase activity; P:auxin biosynthetic process; F:protein binding
Mado-00-G2-K2-002C_E12	265	zinc finger family protein	3.61782E-28	85.65%	C:endomembrane system; F:metal ion binding; C:cytoplasmic membrane-bounded vesicle; C:membrane; F:zinc ion binding; F:protein binding
Mado-00-G2-K2-002C_F01	247	atp-dependent rna	2.08159E-36	91.15%	F:helicase activity; P:auxin biosynthetic process; F:hydrolase activity; F:nucleic acid binding; F:RNA helicase activity; F:nucleotide binding; F:nucleoside-triphosphatase activity; P:embryonic development ending in seed dormancy; F:ATP binding; F:ATP-dependent helicase activity; C:cellular_component
Mado-00-G2-K2-002C_F02	385	chaperone protein	7.65254E-55	95.70%	P:response to stress; C:membrane; C:cytoplasm; F:ATP binding; F:heat shock protein binding; P:response to heat; P:protein folding; F:metal ion binding; C:plasma membrane; F:unfolded protein binding
Mado-00-G2-K2-002C_F03	234	beta-amylase 8	1.61592E-36	92.15%	F:cation binding; P:starch catabolic process; F:hydrolase activity; P:polysaccharide catabolic process; P:carbohydrate metabolic process; F:catalytic activity; P:response to cold; P:maltose biosynthetic process; C:chloroplast stroma; P:metabolic process; F:hydrolase activity, acting on glycosyl bonds; C:plastid; F:beta-amylase activity
Mado-00-G2-K2-002C_F06	293	protein	9.5161E-45	93.35%	
Mado-00-G2-K2-002C_F08	162	wd-40 repeat family protein	7.5491E-18	91.15%	C:heterotrimeric G-protein complex; F:molecular_function; P:biological_process; C:CUL4 RING ubiquitin ligase complex
Mado-00-G2-K2-002C_F11	235	xyloglucan endotransglycosylase	5.39181E-24	94.90%	F:hydrolase activity, hydrolyzing O-glycosyl compounds; C:cell wall; F:transferase activity; P:carbohydrate metabolic process; F:hydrolase activity; C:apoplast; F:xyloglucan:xyloglucosyl transferase activity; P:cellular glucan metabolic process
Mado-00-G2-K2-002C_G02	239	protein	4.40591E-18	66.00%	C:cytoplasm; P:biological_process; F:zinc ion binding
Mado-00-G2-K2-002C_G03	201	unnamed protein product [Vitis vinifera]	0.0260006	68.00%	
Mado-00-G2-K2-002C_G06	220	wak-like kinase	5.32399E-11	64.83%	P:auxin biosynthetic process; F:protein serine/threonine kinase activity; F:2-alkenal reductase activity; F:protein kinase activity; P:protein amino acid phosphorylation; F:nucleotide binding; F:ATP binding; P:oxidation reduction; F:kinase activity; F:oxidoreductase activity
Mado-00-G2-K2-002C_G08	291	protein	3.16053E-48	94.45%	C:peroxisome; P:amine metabolic process; F:quinone binding; P:oxidation reduction; F:amine oxidase activity; F:copper ion binding
Mado-00-G2-K2-002C_G10	180	gras family transcription factor	1.03329E-19	83.10%	P:transcription; P:regulation of transcription
Mado-00-G2-K2-002C_H02	159	gata transcription factor 25	2.12926E-20	75.93%	F:metal ion binding; P:regulation of transcription, DNA-dependent; F:sequence-specific DNA binding; F:zinc ion binding; F:transcription factor activity
Mado-00-G2-K2-002C_H03	339	alcohol dehydrogenase class iii	2.0885E-12	99.25%	P:seed development; P:cell death; C:cytoplasm; F:zinc ion binding; P:ethanol oxidation; P:oxidation reduction; F:S-nitrosoglutathione reductase activity; F:oxidoreductase activity; F:catalytic activity; F:S-(hydroxymethyl)glutathione dehydrogenase activity; F:alcohol dehydrogenase (NAD) activity; P:formaldehyde metabolic process; P:heat acclimation; P:metabolic process; F:metal ion binding; F:binding
Mado-00-G2-K2-002C_H05	167	4 protein	3.85582E-22	95.70%	F:ATP-dependent peptidase activity; F:microtubule-severing ATPase activity; F:serine-type endopeptidase activity; P:auxin biosynthetic process; F:metalloendopeptidase activity; C:membrane; P:protein catabolic process; C:mitochondrion; F:hydrolase activity; F:nucleotide binding; F:nucleoside-triphosphatase activity; F:ATP binding; F:metallopeptidase activity; F:ATPase activity; F:peptidase activity; P:proteolysis; C:plastid
Mado-00-G2-K2-002C_H08	404	neutral invertase	4.54604E-39	96.30%	F:beta-fructofuranosidase activity; C:mitochondrion; P:metabolic process; F:hydrolase activity; F:hydrolase activity, acting on glycosyl bonds; F:catalytic activity; P:biological_process
Mado-00-G2-K2-002C_H10	298	defensin protein	2.26425E-22	85.15%	P:defense response; P:killing of cells of another organism; F:peptidase inhibitor activity; P:defense response to fungus; C:plant-type cell wall; F:peptidase activity; C:extracellular region
Mado-00-G2-K2-002C_H11	175	protein	4.22231E-21	85.20%	F:pyridoxal phosphate binding; P:biosynthetic process; F:transcription factor activity; C:nucleus; F:amino acid binding; F:catalytic activity; P:regulation of transcription, DNA-dependent; P:metabolic process; C:plastid; C:chloroplast
Mado-00-G2-K2-002C_H12	346	rac-gtp binding	2.35567E-35	85.65%	C:mitochondrion; C:mitochondrial outer membrane; F:nucleotide binding; F:GTP binding; P:embryonic development ending in seed dormancy; P:mitochondrion organization; P:embryonic development; C:intracellular; P:mitochondrion transport along microtubule; P:pollen tube growth; P:small GTPase mediated signal transduction; F:calcium ion binding

Mado-00-G2-K2-003C_A06	490	nucleosome chromatin assembly factor group	1.54712E-71	80.50%	F:molecular_function; F:acyltransferase activity; F:transferase activity; F:histone acetyltransferase activity; P:biological_process; F:protein binding; C:CUL4 RING ubiquitin ligase complex
Mado-00-G2-K2-003C_A10	267	tpl protein binding protein homodimerization transcription repressor	1.72494E-38	90.80%	F:protein homodimerization activity; C:nucleus; P:response to auxin stimulus; P:jasmonic acid mediated signaling pathway; P:xylem and phloem pattern formation; F:transcription repressor activity; F:protein binding; C:cytosol; P:primary shoot apical meristem specification
Mado-00-G2-K2-003C_A11	237	fkbp-rapamycin associated	1.11523E-37	96.90%	F:kinase activity; F:binding; F:transferase activity; P:embryonic development ending in seed dormancy; F:phosphotransferase activity, alcohol group as acceptor; F:transferase activity, transferring phosphorus-containing groups; F:protein binding; F:obs-phosphatidylinositol-3-kinase activity
Mado-00-G2-K2-003C_B02	277	seed specific protein bn15d1b	5.17436E-11	61.05%	C:mitochondrion; F:molecular_function; P:biological_process; C:cellular_component
Mado-00-G2-K2-003C_B09	442	ramosa 1 enhancer locus 2	1.42617E-40	82.05%	
Mado-00-G2-K2-003C_B12	393	6-phosphofructo-2-kinase family expressed	4.13372E-64	93.20%	F:transferase activity; F:fructose-2,6-bisphosphate 2-phosphatase activity; F:carbohydrate binding; F:hydrolase activity; F:ATP binding; F:kinase activity; P:carbohydrate metabolic process; F:catalytic activity; P:metabolic process; P:fructose 2,6-bisphosphate metabolic process; C:cytosol; F:6-phosphofructo-2-kinase activity; P:fructose metabolic process
Mado-00-G2-K2-003C_C07	231	mal d	2.50733E-29	96.15%	P:response to biotic stimulus; P:defense response
Mado-00-G2-K2-003C_C11	148	gonidia forming protein	3.80614E-17	91.50%	P:protein folding; F:heat shock protein binding; C:cellular_component; F:DNA binding
Mado-00-G2-K2-003C_D02	138	4-hydroxyphenylpyruvate dioxygenase	4.07769E-16	94.50%	F:4-hydroxyphenylpyruvate dioxygenase activity; C:mitochondrion; F:oxidoreductase activity, acting on single donors with incorporation of molecular oxygen, incorporation of two atoms of oxygen; C:cytoplasm; P:carotenoid biosynthetic process; P:vitamin E biosynthetic process; P:oxidation reduction; P:tyrosine catabolic process; P:plastoquinone biosynthetic process; F:oxidoreductase activity; P:aromatic amino acid family metabolic process; F:metal ion binding; C:cytosol; P:L-phenylalanine catabolic process: C:plastid: C:chloroplast
Mado-00-G2-K2-003C_D05	414	hexokinase 6	1.06419E-40	81.35%	F:transferase activity; P:auxin biosynthetic process; C:mitochondrion; F:nucleotide binding; F:ATP binding; P:response to osmotic stress; F:kinase activity; P:carbohydrate metabolic process; P:glycolysis; P:response to cold; P:response to salt stress; F:glucokinase activity; F:hexokinase activity; F:phosphotransferase activity, alcohol group as acceptor; F:fructokinase activity; C:plastid
Mado-00-G2-K2-003C_D09	215	delta-12 oleate desaturase	3.12307E-32	99.70%	F:delta12-fatty acid dehydrogenase activity; P:lipid metabolic process; P:oxidation reduction; F:oxidoreductase activity; C:membrane
Mado-00-G2-K2-003C_E03	178	cornichon family protein	5.90008E-7	71.10%	C:endomembrane system; F:molecular_function; C:cytoplasmic membrane-bounded vesicle; C:membrane; P:intracellular signaling pathway
Mado-00-G2-K2-003C_E04	578	alpha beta fold family protein	1.24554E-19	61.70%	C:plastid; F:hydrolase activity; F:polyneuridine-aldehyde esterase activity
Mado-00-G2-K2-003C_E08	126	t-complex protein 1 subunit epsilon	4.5645E-15	96.60%	P:auxin biosynthetic process; P:cellular protein metabolic process; F:nucleotide binding; C:cytoplasm; F:ATP binding; F:protein binding; P:protein folding; C:plasma membrane; F:unfolded protein binding
Mado-00-G2-K2-003C_E10	168	dead deah box helicase	8.03284E-12	94.50%	F:helicase activity; P:auxin biosynthetic process; F:hydrolase activity; F:nucleic acid binding; F:RNA helicase activity; F:nucleotide binding; F:nucleoside-triphosphatase activity; P:embryonic development ending in seed dormancy; F:ATP binding; F:ATP-dependent helicase activity; C:cellular_component
Mado-00-G2-K2-003C_F02	292	molybdenum cofactor sulfurase family protein	6.63612E-22	69.10%	F:Mo-molybdopterin cofactor sulfurase activity; C:chloroplast; F:molybdenum ion binding; F:catalytic activity; P:biological_process; F:pyridoxal phosphate binding; C:cellular_component
Mado-00-G2-K2-003C_F04	340	cyclic nucleotide and calmodulin-regulated ion channel	1.63195E-57	97.55%	F:cGMP-dependent protein kinase activity; P:protein amino acid phosphorylation; F:voltage-gated potassium channel activity; F:ion channel activity; F:ATP binding; C:integral to membrane; C:membrane; F:protein kinase activity; F:cyclic nucleotide binding; P:transmembrane transport; P:auxin biosynthetic process; F:nucleotide binding; P:potassium ion transport; P:ion transport; P:transport; F:calmodulin binding; F:potassium channel activitv: F:transferase activitv; C:plasma membrane
Mado-00-G2-K2-003C_F05	141	membrane protein	4.21639E-5	84.00%	C:plastid; C:membrane
Mado-00-G2-K2-003C_F07	276	glyoxalase i	5.69445E-26	91.30%	F:metal ion binding; F:lactoylglutathione lyase activity; F:lyase activity
Mado-00-G2-K2-003C_F08	194	PREDICTED: hypothetical protein [Vitis vinifera]	0.00529089	62.67%	
Mado-00-G2-K2-003C_F09	267	two-pore calcium channel	1.01831E-14	84.05%	F:calcium channel activity; C:vacuolar membrane; P:seed germination; F:ion channel activity; F:voltage-gated calcium channel activity; F:voltage-gated ion channel activity; C:integral to membrane; C:membrane; C:plant-type vacuole; P:regulation of stomatal movement; F:calcium ion binding; P:transmembrane transport; P:calcium-mediated signaling; P:calcium ion transport; P:defense response; P:ion transport; P:transport; C:plasma membrane
Mado-00-G2-K2-003C_F11	437	une5 (unfertilized embryo sac 5) protein disulfide isomerase	1.75071E-59	92.05%	C:plant-type cell wall; C:cytoplasmic membrane-bounded vesicle; F:electron carrier activity; F:protein disulfide isomerase activity; P:embryo sac development; P:pollen tube development; P:embryonic development ending in seed dormancy; P:response to endoplasmic reticulum stress; C:endoplasmic reticulum lumen; F:isomerase activity; P:electron transport chain; C:endoplasmic reticulum; P:cell redox homeostasis; P:double fertilization forming a zygote and endosperm; C:plasma membrane
Mado-00-G2-K2-003C_G06	200	pfkb-type carbohydrate kinase family protein	1.9212E-21	80.20%	F:kinase activity; C:chloroplast stroma; F:transferase activity; C:chloroplast; P:D-ribose metabolic process; F:ribokinase activity
Mado-00-G2-K2-003C_G07	125	ankyrin-like protein	3.50565E-15	94.25%	C:membrane; C:Golgi apparatus; P:biological_process
Mado-00-G2-K2-003C_G10	257	cytochrome p450 probable 6-deoxocathasterone to 6-deoxoteasterone or cathasterone to teasterone	5.08985E-38	87.20%	P:response to UV-B; F:heme binding; C:cytoplasmic membrane-bounded vesicle; F:iron ion binding; F:electron carrier activity; P:tapetal cell differentiation; P:brassinosteroid homeostasis; P:oxidation reduction; P:pollen exine formation; F:oxidoreductase activity; F:taxane 13-alpha-hydroxylase activity; P:positive regulation of flower development; F:metal ion binding; F:oxygen binding; F:monooxygenase activity; P:unidimensional cell growth; P:brassinosteroid biosynthetic process
Mado-00-G2-K2-003C_H05	123	binding protein	3.3955E-10	84.45%	F:binding; C:chloroplast; P:RNA processing; P:biological_process; C:intracellular
Mado-00-G2-K2-003C_H06	242	zinc ion binding protein	5.17467E-27	70.2%	C:phragmoplast; C:cytosol; F:zinc ion binding; C:intracellular; C:nucleus
Mado-00-G2-K2-003C_H08	231	autophagy 4a	8.34681E-25	85.00%	C:autophagic vacuole; P:protein transport; C:vacuolar lumen; F:cysteine-type carboxypeptidase activity; F:hydrolase activity; P:transport; C:cytoplasm; P:autophagy; F:APG8-specific protease activity; F:cysteine-type peptidase activity; F:peptidase activity; F:protein binding; C:chloroplast
Mado-00-G2-K2-003C_H09	223	branched-chain amino acid	2.21384E-33	94.65%	C:mitochondrion; P:branched chain family amino acid metabolic process; F:branched-chain-amino-acid transaminase activity; C:chloroplast; F:transferase activity; P:metabolic process; F:catalytic activity; F:transaminase activity
Mado-00-G2-K2-003C_H11	156	adenylate translocator	6.87129E-19	97.10%	F:structural constituent of cytoskeleton; C:mitochondrial inner membrane; C:vacuole; C:chloroplast; P:developmental growth; C:mitochondrial envelope; C:nucleus; F:transporter activity; C:integral to membrane; C:membrane; C:cell wall; F:ATP:ADP antiporter activity; F:copper ion binding; P:cytoskeleton organization; P:root hair elongation; P:transmembrane transport; C:mitochondrion; F:binding; C:nucleolus; P:transport; P:purine nucleotide transport; C:cytoskeleton; C:plasma membrane
Mado-00-G2-K2-003C_H12	192	protein disulfide isomerase like protein	2.06615E-23	87.35%	P:cell redox homeostasis; C:cytoplasmic membrane-bounded vesicle; F:protein disulfide isomerase activity; F:transferase activity; C:endoplasmic reticulum; F:dolichyl-diphosphooligosaccharide-protein glycotransferase activity; F:transferase activity, transferring glycosyl groups; F:isomerase activity
Mado-00-G2-K2-004_A01	169	conserved hypothetical protein [Ricinus communis]	1.61481E-20	84.80%	F:binding; C:chloroplast; C:membrane; P:biological_process; P:response to oxidative stress
Mado-00-G2-K2-004_A02	240	peroxidase 12	1.23452E-20	77.45%	F:metal ion binding; P:oxidation reduction; C:cytoplasmic membrane-bounded vesicle; F:oxidoreductase activity; F:heme binding; P:response to oxidative stress; F:peroxidase activity

	Mado-00-G2-K2-004_A09	185 chloroplast alpha-glucan water	1.14487E-21	84.75%	F:transferase activity; F:carbohydrate binding; P:phosphorylation; P:starch catabolic process; P:starch metabolic process; P:protein amino acid autophosphorylation; F:ATP binding; F:kinase activity; F:carbohydrate kinase activity; F:phosphoglucan, water dikinase activity; P:carbohydrate metabolic process; F:catalytic activity; C:chloroplast stroma; C:plastid; C:chloroplast
	Mado-00-G2-K2-004_A10	148 protein	1.49672E-13	79.20%	F:transferase activity; F:carbohydrate binding; P:phosphorylation; P:starch catabolic process; P:starch metabolic process; P:protein amino acid autophosphorylation; F:ATP binding; F:kinase activity; F:carbohydrate kinase activity; F:phosphoglucan, water dikinase activity; P:carbohydrate metabolic process; F:catalytic activity; C:chloroplast stroma; C:plastid; C:chloroplast
	Mado-00-G2-K2-004_A12	319 zinc finger family protein	4.27143E-53	89.15%	F:metal ion binding; F:zinc ion binding; F:protein binding
	Mado-00-G2-K2-004_B04	237 brassinosteroid-regulated protein bru1	5.38585E-16	89.75%	F:transferase activity; F:xyloglucan:xyloglucosyl transferase activity; F:hydrolase activity; F:transferase activity, transferring glycosyl groups; P:cellular glucan metabolic process; C:apoplast; P:carbohydrate metabolic process; F:hydrolase activity, hydrolyzing O-glycosyl compounds; P:metabolic process; C:cell wall; F:hydrolase activity, acting on glycosyl bonds
	Mado-00-G2-K2-004_B08	310 digalactosyldiacylglycerol synthase expressed	8.49799E-14	81.85%	F:transferase activity; P:galactolipid biosynthetic process; P:biosynthetic process; P:nodulation; C:membrane; C:plastid outer membrane; F:UDP-glycosyltransferase activity; F:transferase activity, transferring glycosyl groups; C:chloroplast outer membrane; P:cellular response to phosphate starvation; C:peribacteroid membrane; F:UDP-galactosyltransferase activity; F:digalactosyldiacylglycerol synthase activity; P:glycolipid biosynthetic process; C:plastid; C:chloroplast
	Mado-00-G2-K2-004_B10	289 atp binding	1.48719E-13	69.91%	
	Mado-00-G2-K2-004_C02	292 galactinol synthase 1	4.72279E-44	93.85%	P:carbohydrate biosynthetic process; F:transferase activity; F:inositol 3-alpha-galactosyltransferase activity; F:transferase activity, transferring hexosyl groups; C:cellular_component; F:transferase activity, transferring glycosyl groups
	Mado-00-G2-K2-004_C10	184 protein	1.14487E-21	87.30%	F:DNA binding; F:transcription regulator activity; F:nucleic acid binding; F:zinc ion binding; C:cellular_component; P:regulation of transcription, DNA-dependent; F:protein binding; F:transcription coactivator activity; F:binding
	Mado-00-G2-K2-004_C12	442 aldo keto reductase family protein	4.25443E-69	86.50%	F:steroid dehydrogenase activity; C:nucleus; F:aldo-keto reductase activity; F:aldehyde reductase activity; P:oxidation reduction; P:response to water deprivation; F:oxidoreductase activity; P:response to cold; P:response to salt stress; P:response to cadmium ion; C:cytosol
	Mado-00-G2-K2-004_D03	301 aldose reductase	1.19068E-39	91.25%	F:steroid dehydrogenase activity; C:nucleus; F:aldo-keto reductase activity; F:aldehyde reductase activity; P:oxidation reduction; P:response to water deprivation; F:oxidoreductase activity; P:response to cold; P:response to salt stress; C:cytosol
	Mado-00-G2-K2-004_D12	286 protein	6.47427E-17	73.45%	C:mitochondrion; P:rRNA transcription; P:cell proliferation; F:transcription factor activity; F:transcription factor binding
	Mado-00-G2-K2-004_E09	492 elongation factor 1-	3.41491E-87	98.15%	F:transferase activity; F:sulfate adenylyltransferase (ATP) activity; P:translational elongation; F:nucleotide binding; C:cytoplasm; F:GTP binding; F:nucleotidyltransferase activity; F:translation elongation factor activity; F:GTPase activity
	Mado-00-G2-K2-004_E10	243 vf14-3-3d protein	2.49043E-5	77.00%	F:protein domain specific binding
	Mado-00-G2-K2-004_E11	333 nhl repeat-containing protein	1.6598E-25	66.45%	C:endomembrane system; F:molecular_function; P:biological_process
	Mado-00-G2-K2-004_F03	318 gras family transcription factor	2.28072E-30	78.55%	P:transcription; P:regulation of transcription; F:transcription factor activity
	Mado-00-G2-K2-004_F07	243 prolyl carboxypeptidase like expressed	1.35393E-35	90.85%	P:proteolysis; C:cytoplasmic membrane-bounded vesicle; F:carboxypeptidase activity; F:serine-type peptidase activity; F:hydrolase activity
	Mado-00-G2-K2-004_F09	468 proton-dependent oligopeptide transport family protein	2.31473E-67	74.80%	P:oligopeptide transport; C:integral to membrane; C:membrane; P:transport; F:transporter activity
	Mado-00-G2-K2-004_F11	309 quinone oxidoreductase	6.90359E-40	82.75%	C:stromule; C:thylakoid; C:apoplast; F:zinc ion binding; P:oxidation reduction; F:oxidoreductase activity; F:catalytic activity; F:NADPH:quinone reductase activity; P:response to cold; C:chloroplast envelope; C:chloroplast stroma; P:metabolic process; C:chloroplast; F:binding
	Mado-00-G2-K2-004_G01	249 binding protein	2.3789E-24	79.69%	F:binding
	Mado-00-G2-K2-004_G05	265 protein	9.87515E-18	65.80%	C:endomembrane system; F:nucleic acid binding; P:transcription initiation from RNA polymerase II promoter; F:translation initiation factor activity; F:zinc ion binding; C:transcription factor TFIIE complex; F:RNA polymerase II transcription factor activity; F:transcription initiation factor activity
	Mado-00-G2-K2-004_G09	177 protein kinase	9.37604E-21	86.30%	C:cytoplasmic membrane-bounded vesicle; C:integral to membrane; C:membrane; F:protein kinase activity; P:protein amino acid phosphorylation; F:ATP binding; F:kinase activity; F:receptor activity; F:protein binding
	Mado-00-G2-K2-004_G10	143 protein	1.26364E-17	92.30%	F:UDP-arabinose 4-epimerase activity; F:coenzyme binding; P:cellular metabolic process; C:Golgi apparatus; P:galactose metabolic process; F:catalytic activity; P:nucleotide-sugar metabolic process; F:isomerase activity; P:plant-type cell wall biogenesis; P:arabinose biosynthetic process; C:endomembrane system; P:metabolic process; F:UDP-glucose 4-epimerase activity; F:binding
	Mado-00-G2-K2-004_G11	194 protein	4.61359E-15	73.25%	P:regulation of transcription, DNA-dependent; C:mitochondrion; F:sequence-specific DNA binding; F:DNA binding; C:intracellular; C:nucleus
	Mado-00-G2-K2-004_H02	178 constans 1	1.23024E-4	69.12%	F:zinc ion binding; C:intracellular
	Mado-00-G2-K2-004_H04	277 cyclin family protein	6.92878E-40	90.35%	P:regulation of cell cycle; C:mitochondrion; F:cyclin-dependent protein kinase regulator activity; P:cell cycle; F:cyclin-dependent protein kinase activity; C:nucleus
	Mado-00-G2-K2-004_H06	423 math domain containing expressed	2.67346E-39	62.10%	C:endomembrane system; F:molecular_function; P:biological_process
	Mado-00-G2-K2-004_H09	514 pseudo response regulator	6.452E-23	56.89%	F:kinase activity; P:two-component signal transduction system (phosphorelay); P:regulation of transcription, DNA-dependent; F:two-component response regulator activity
	Mado-00-G2-K2-004_H10	347 cinnamoyl- reductase	1.93154E-29	80.50%	F:dihydrokaempferol 4-reductase activity; F:coenzyme binding; P:cellular metabolic process; F:cinnamoyl-CoA reductase activity; P:oxidation reduction; P:lignin biosynthetic process; F:3-beta-hydroxy-delta5-steroid dehydrogenase activity; F:oxidoreductase activity; F:catalytic activity; C:cellular_component; P:metabolic process; P:response to cadmium ion; P:steroid biosynthetic process; F:binding
K1-G1	Mado-00-K1-G1-Contig1	616 protein	2.11738E-58	86.35%	P:oxidation reduction; F:aromatase activity
	Mado-00-K1-G1-Contig2	500 protein	2.775E-89	95.25%	F:protein binding; P:protein catabolic process; P:photomorphogenesis; C:signalosome; P:cullin deneddylation
	Mado-00-K1-G1-Contig3	484 metallothionein-like protein	4.46313E-26	88.30%	F:metal ion binding
	Mado-00-K1-G1-Contig4	309 chloroplast-localized ptr -binding protein1	1.39487E-40	88.70%	C:plastid outer membrane; P:G-protein coupled receptor protein signaling pathway; P:protein import into chloroplast thylakoid membrane; P:thylakoid membrane organization; F:protein binding; C:chloroplast thylakoid membrane; C:chloroplast stroma; P:photosystem II assembly; P:protein import into chloroplast stroma; P:sugar mediated signaling pathway; C:plastid inner membrane; C:stromule
	Mado-00-K1-G1-Contig5	306 senescence-associated protein	9.36926E-45	92.00%	
	Mado-00-K1-G1-Contig6	242 transcription initiation factor iif subunit	3.71022E-33	88.25%	F:translation initiation factor activity; P:transcription initiation from RNA polymerase II promoter; C:mitochondrion; F:ATP binding; C:transcription factor TFIIF complex; F:catalytic activity; F:RNA polymerase II transcription factor activity
	Mado-00-K1-G1-Contig7	226 ubiquitin conjugating enzyme 2	4.61353E-31	95.50%	F:RNA-directed DNA polymerase activity; P:RNA-dependent DNA replication; F:RNA binding; P:ubiquitin-dependent protein catabolic process; F:ubiquitin-protein ligase activity; P:regulation of protein metabolic process; P:post-translational protein modification
	Mado-00-K1-G1-Contig8	223 ap2 domain class transcription factor	5.67189E-21	83.85%	F:transcription factor activity; C:nucleus; P:regulation of transcription, DNA-dependent
	Mado-00-K1-G1-Contig9	514 something about silencing protein	9.91081E-48	62.00%	F:binding; P:cellular process; P:macromolecule metabolic process

Mado-00-K1-G1-Contig10	478	pi starvation-induced protein	1.77359E-27	83.45%	F:identical protein binding; F:serine-type endopeptidase activity; C:cytoplasmic membrane-bounded vesicle; C:plasma membrane; P:negative regulation of catalytic activity
Mado-00-K1-G1-Contig11	441	60s ribosomal protein	9.50318E-69	93.85%	C:vacuole; F:structural constituent of ribosome; C:plasma membrane; F:RNA binding; C:cytosolic large ribosomal subunit; P:translation
Mado-00-K1-G1-Contig12	438	protein	1.16425E-79	95.50%	F:translation elongation factor activity; P:response to cold; F:GTP binding; F:copper ion binding; C:plasma membrane; C:chloroplast; C:cytosol; P:translation; F:GTPase activity
Mado-00-K1-G1-Contig13	415	gtk16 protein	8.78995E-19	84.15%	C:membrane; C:cytoplasmic membrane-bounded vesicle
Mado-00-K1-G1-Contig14	396	atp binding protein	1.062E-27	87.90%	P:protein amino acid phosphorylation; C:endomembrane system; F:protein tyrosine kinase activity; F:ATP binding; C:mitochondrion
Mado-00-K1-G1-Contig15	386	protein	2.02868E-39	72.35%	C:nucleolus; C:ribosome; C:chloroplast envelope; F:structural constituent of ribosome; F:RNA binding; C:membrane; P:translation; P:RNA processing
Mado-00-K1-G1-Contig16	386	protein	1.11318E-37	66.82%	C:membrane; C:cytoplasmic membrane-bounded vesicle; C:endoplasmic reticulum
Mado-00-K1-G1-Contig17	369	protein disulfide	1.58103E-47	91.80%	P:cell redox homeostasis; C:cytoplasmic membrane-bounded vesicle; C:endoplasmic reticulum; F:protein disulfide isomerase activity
Mado-00-K1-G1-Contig18	363	rna helicase	5.33199E-24	90.10%	P:auxin biosynthetic process; F:ATP-dependent helicase activity; F:nucleic acid binding; F:ATP binding; C:mitochondrion
Mado-00-K1-G1-Contig20	347	dehydrin	1.69675E-25	75.95%	P:response to water; P:response to stress
Mado-00-K1-G1-Contig21	343	defensin 1	9.12997E-14	85.15%	F:peptidase inhibitor activity; F:peptidase activity; C:plant-type cell wall; C:cytoplasmic membrane-bounded vesicle; P:defense response to fungus; C:extracellular region; P:killing of cells of another organism
Mado-00-K1-G1-Contig22	340	heat shock	2.28875E-51	93.80%	P:response to water deprivation; C:mitochondrion; P:embryonic development ending in seed dormancy; P:protein folding; P:response to salt stress; P:response to chlorate; P:response to heat; F:ATP binding; C:chloroplast stroma; F:unfolded protein binding; P:de-etiolation; C:plasma membrane
Mado-00-K1-G1-Contig23	339	protein	4.35662E-18	57.53%	F:zinc ion binding; C:intracellular
Mado-00-K1-G1-Contig24	338	c2 domain-containing protein	8.83246E-11	79.6875%	C:membrane; P:phosphoinositide-mediated signaling; P:calcium-mediated signaling; P:pollen maturation
Mado-00-K1-G1-Contig25	332	rare cold inducible protein	3.26816E-13	92.35%	P:response to abscisic acid stimulus; C:integral to membrane; P:response to cold; P:hyperosmotic salinity response
Mado-00-K1-G1-Contig26	317	aconitate hydratase 1	2.60826E-50	94.85%	F:aconitate hydratase activity; P:metabolic process; F:4 iron, 4 sulfur cluster binding; C:mitochondrion; C:plastid
Mado-00-K1-G1-Contig27	297	60s ribosomal protein l36-2	1.12707E-32	94.45%	F:structural constituent of ribosome; C:plasma membrane; C:cytosolic large ribosomal subunit; P:translation
Mado-00-K1-G1-Contig30	273	phd f-box containing	1.96507E-34	95.55%	F:DNA binding; F:methylated histone residue binding; C:nucleus; F:zinc ion binding; P:regulation of transcription, DNA-dependent
Mado-00-K1-G1-Contig32	245	fiber protein fb15	5.02047E-14	84.07%	C:mitochondrion; C:plastid
Mado-00-K1-G1-Contig33	242	cysteine proteinase inhibitor	1.28111E-17	87.70%	F:peptidase activity; F:cysteine-type endopeptidase inhibitor activity
Mado-00-K1-G1-Contig35	208	sterol c-14 reductase	3.71426E-25	80.75%	C:cytoplasmic membrane-bounded vesicle; P:embryonic development ending in seed dormancy; C:membrane; F:delta14-sterol reductase activity; P:sterol biosynthetic process
Mado-00-K1-G1-Contig36	203	ribulose- -bisphosphate carboxylase oxygenase large subunit	1.67447E-33	100.00%	P:reductive pentose-phosphate cycle; F:ribulose-bisphosphate carboxylase activity; F:monooxygenase activity; P:oxidation reduction; F:magnesium ion binding; C:chloroplast
Mado-00-K1-G1-Contig38	195	delta-12 oleate desaturase	3.89484E-22	83.65%	P:oxidation reduction; P:lipid metabolic process; F:phosphatidylcholine desaturase activity
Mado-00-K1-G1-Contig39	190	conserved hypothetical protein [Ricinus communis]	2.54115E-21	84.90%	C:membrane; F:binding; C:chloroplast; P:response to oxidative stress
Mado-00-K1-G1-Contig40	186	terpene synthase-like terpenoid synthase	2.64439E-10	73.00%	F:metal ion binding; F:lyase activity
Mado-00-K1-G1-Contig41	185	multidrug resistance protein abc transporter family	3.56903E-23	85.80%	P:transmembrane transport; F:sulfonylurea receptor activity; P:auxin biosynthetic process; C:mitochondrion; P:response to salt stress; C:integral to membrane; F:ATPase activity, coupled to transmembrane movement of substances; P:oxidation reduction; F:2-alkenal reductase activity; C:vacuolar membrane; F:ATP binding; P:cellular potassium ion homeostasis; C:plasma membrane
Mado-00-K1-G1-Contig42	175	transcriptional corepressor	7.20592E-29	92.55%	P:embryonic development; F:DNA binding; F:transcription cofactor activity; F:molecular adaptor activity; P:regulation of flower development; F:protein heterodimerization activity; C:plastid; P:ovule development
Mado-00-K1-G1-Contig46	112	mybr domain class transcription factor	3.81108E-9	100.00%	
Mado-00-K1-G1-001_A02	178	conserved hypothetical protein [Ricinus communis]	6.54184E-6	69.67%	F:zinc ion binding; C:intracellular; F:protein binding
Mado-00-K1-G1-001_A03	284	at3g16920 k14a17_4	1.39295E-43	90.65%	P:multidimensional cell growth; P:root epidermal cell differentiation; P:chitin catabolic process; P:cell wall macromolecule catabolic process; P:response to water deprivation; P:response to salt stress; P:response to cytokinin stimulus; P:response to heat; P:response to nitrate; P:lignin biosynthetic process; F:chitinase activity; P:regulation of salicylic acid metabolic process
Mado-00-K1-G1-001_A04	141	protein transport factor	4.9949E-6	96.07%	C:COPII vesicle coat; F:protein binding; P:intracellular protein transport; P:ER to Golgi vesicle-mediated transport; F:zinc ion binding; C:plastid
Mado-00-K1-G1-001_A08	226	c2 domain-containing protein	8.18151E-12	77.50%	C:chloroplast
Mado-00-K1-G1-001_B03	228	PREDICTED: hypothetical protein [Vitis vinifera]	8.73183E-6	82.00%	P:auxin biosynthetic process; F:ATP-dependent helicase activity; F:RNA binding; F:ATP binding; C:nucleus
Mado-00-K1-G1-001_B10	283	beta-galactosidase like protein	2.53856E-45	90.35%	P:carbohydrate metabolic process; C:cytoplasmic membrane-bounded vesicle; F:protein binding; F:beta-galactosidase activity; F:sugar binding; F:cation binding
Mado-00-K1-G1-001_B11	226	cbl-interacting serine threonine-protein	5.29082E-19	80.20%	P:auxin biosynthetic process; F:calmodulin-dependent protein kinase activity; P:protein amino acid phosphorylation; F:protein binding; P:defense response to fungus; F:ATP binding; P:signal transduction
Mado-00-K1-G1-001_C06	179	5-enolpyruvylshikimate-3-phosphate synthase	1.18944E-15	94.20%	F:3-phosphoshikimate 1-carboxyvinyltransferase activity; P:aromatic amino acid family biosynthetic process; C:chloroplast
Mado-00-K1-G1-001_C07	212	coiled-coil domain-containing	1.35621E-35	96.30%	F:molecular_function; P:biological_process; C:cellular_component
Mado-00-K1-G1-001_C08	358	class iii chitinase	2.55354E-58	79.95%	F:chitinase activity; C:extracellular space; P:chitin catabolic process; F:cation binding; C:cytoplasmic membrane-bounded vesicle
Mado-00-K1-G1-001_C09	501	phytocyanin	1.21535E-31	60.75%	F:electron carrier activity; F:copper ion binding
Mado-00-K1-G1-001_C11	219	low-temperature-induced 65 kd protein	7.9781E-7	56.67%	P:abscisic acid mediated signaling pathway; P:response to water deprivation; P:response to salt stress; P:response to cold
Mado-00-K1-G1-001_D08	509	protein	1.27316E-39	87.25%	F:ATPase activity, coupled to transmembrane movement of ions, phosphorylative mechanism; P:ATP biosynthetic process; F:ATP binding; C:membrane
Mado-00-K1-G1-001_D09	149	protein	9.70662E-21	94.20%	C:ribosome; P:carbohydrate metabolic process; F:polygalacturonase activity; F:structural constituent of ribosome; P:translation
Mado-00-K1-G1-001_E01	441	dna-binding family protein	6.65792E-30	64.63%	C:nucleus; F:DNA binding
Mado-00-K1-G1-001_E02	189	kinase family protein	1.49322E-13	86.00%	P:auxin biosynthetic process; P:protein amino acid phosphorylation; F:RNA-directed DNA polymerase activity; P:RNA-dependent DNA replication; F:RNA binding; F:ATP binding; F:protein serine/threonine kinase activity

Mado-00-K1-G1-001_E03	164	zinc finger protein	1.92967E-13	83.25%	F:zinc ion binding; C:cytoplasm
Mado-00-K1-G1-001_E05	191	sh3 domain-containing protein 2	7.17793E-8	77.25%	C:cytoplasm; F:clathrin binding
Mado-00-K1-G1-001_F02	136	tho complex subunit 4	3.83942E-14	94.10%	F:nucleic acid binding; F:nucleotide binding
Mado-00-K1-G1-001_F06	482	imbibition protein homolog	2.44086E-61	76.25%	F:transferase activity, transferring glycosyl groups; F:hydrolase activity, hydrolyzing O-glycosyl compounds
Mado-00-K1-G1-001_G04	338	arginine serine-rich splicing	1.64653E-49	85.45%	C:spliceosomal complex; F:RNA binding; F:protein binding; C:nuclear speck; P:nuclear mRNA splicing, via spliceosome; F:nucleotide binding
Mado-00-K1-G1-001_G05	600	atprep2 (arabidopsis thaliana presequence protease 2) catalytic metal ion binding metalloendopeptidase metallopeptidase zinc ion binding	2.46872E-93	85.70%	F:metalloendopeptidase activity; P:response to cadmium ion; P:protein maturation by peptide bond cleavage; C:chloroplast stroma; F:zinc ion binding; C:apoplast; C:mitochondrion
Mado-00-K1-G1-001_G08	378	thaumatin-like protein	1.97166E-50	90.40%	C:extracellular region; P:defense response to fungus; P:killing of cells of another organism
Mado-00-K1-G1-001_G11	315	PREDICTED: hypothetical protein [Vitis vinifera]	4.31923E-37	80.45%	C:vacuole; C:plastid; C:plasma membrane
Mado-00-K1-G1-001_G12	307	tubulin alpha	4.34211E-50	100.00%	P:microtubule-based movement; F:GTP binding; P:protein polymerization; F:structural molecule activity; F:GTPase activity; C:microtubule
Mado-00-K1-G1-001_H01	350	protein	6.0255E-15	97.60%	C:cytoplasm; P:auxin biosynthetic process; P:protein folding; F:ATP binding; F:unfolded protein binding; C:membrane
Mado-00-K1-G1-001_H02	320	sentrin sumo-specific	2.61062E-34	65.00%	F:NEDD8-specific protease activity; P:proteolysis
Mado-00-K1-G1-001_H04	265	armadillo beta-catenin repeat family protein	9.26868E-16	83.90%	F:binding
Mado-00-K1-G1-001_H08	273	erd15 protein	2.04773E-7	67.33%	P:response to high light intensity; P:response to water deprivation; C:cytoplasm; P:response to bacterium; F:protein binding
Mado-00-K1-G1-001_H10	375	acetolactate synthase small subunit	3.70549E-14	89.65%	F:amino acid binding; F:acetolactate synthase activity; P:branched chain family amino acid biosynthetic process; C:plastid
Mado-00-K1-G1-002_A01	311	protein	1.01199E-33	88.10%	C:endoplasmic reticulum; C:Golgi apparatus; P:intracellular protein transport; F:binding; F:transporter activity
Mado-00-K1-G1-002_A05	163	beta- -glucanase	9.91047E-10	86.26%	F:transferase activity; P:carbohydrate metabolic process; F:hydrolase activity, hydrolyzing O-glycosyl compounds; F:cation binding
Mado-00-K1-G1-002_A06	482	eif4-gamma eif5 eif2-epsilon domain-containing protein	4.50018E-23	94.80%	P:regulation of translational initiation; F:translation initiation factor activity; C:mitochondrion
Mado-00-K1-G1-002_A08	234	nucleolysin tia-	1.3718E-35	90.70%	F:nucleotide binding; F:mRNA 3'-UTR binding
Mado-00-K1-G1-002_A09	252	della protein	8.44257E-41	82.95%	P:regulation of transcription
Mado-00-K1-G1-002_A12	360	brassinosteroid insensitive 1-associated receptor kinase 1	2.71754E-60	96.65%	P:auxin biosynthetic process; C:integral to membrane; P:transmembrane receptor protein tyrosine kinase signaling pathway; C:cytoplasmic membrane-bounded vesicle; F:ATP binding; F:protein binding; F:MAP kinase kinase kinase activity; C:endomembrane system; P:unidimensional cell growth; P:plant-type cell wall organization; F:transmembrane receptor protein tyrosine kinase activity; C:plasma membrane; P:protein amino acid phosphorylation
Mado-00-K1-G1-002_B04	287	ost3 ost6 family protein	3.678E-20	80.90%	C:endomembrane system; C:cytoplasmic membrane-bounded vesicle; C:endoplasmic reticulum; F:oligosaccharide transmembrane transporter activity; C:chloroplast; C:plasma membrane
Mado-00-K1-G1-002_B06	347	eukaryotic translation initiation factor 2 gamma expressed	4.14065E-56	96.35%	F:GTPase activity; F:translation initiation factor activity; F:GTP binding
Mado-00-K1-G1-002_B07	320	protein	2.88638E-33	72.65%	F:protein binding
Mado-00-K1-G1-002_B10	220	lung seven transmembrane receptor family expressed	2.72302E-31	85.70%	C:endomembrane system; C:integral to membrane
Mado-00-K1-G1-002_B11	346	cop1-interacting protein 7 -like	4.18895E-16	89.67%	
Mado-00-K1-G1-002_B12	248	aminophospholipid atpase	1.59829E-36	84.50%	P:root development; C:mitochondrion; C:integral to membrane; F:ATPase activity, coupled to transmembrane movement of ions, phosphorylative mechanism; P:Golgi vesicle budding; P:ATP biosynthetic process; F:ATP binding; P:phospholipid transport; F:magnesium ion binding; F:phospholipid-translocating ATPase activity; P:shoot development; C:Golgi apparatus
Mado-00-K1-G1-002_C03	358	protein	4.08626E-48	71.30%	C:Golgi apparatus; F:transferase activity; C:mitochondrion
Mado-00-K1-G1-002_C04	178	prohibitin	9.08614E-24	98.90%	P:cell growth; C:mitochondrion; C:nucleolus; P:response to salt stress; P:cell division; C:cytoplasmic membrane-bounded vesicle; P:response to auxin stimulus; P:mitochondrion organization; P:lateral root development; P:response to nitric oxide; C:vacuole; C:respiratory chain complex I
Mado-00-K1-G1-002_C05	244	rna-binding region rnp-1 and splicing factor pwi family member protein	9.14945E-16	63.00%	C:plastid; F:nucleic acid binding; F:nucleotide binding; P:mRNA processing
Mado-00-K1-G1-002_C10	365	protein	3.82116E-22	71.00%	F:ubiquitin-protein ligase activity; F:zinc ion binding; F:protein binding; P:plant-type hypersensitive response; C:plasma membrane
Mado-00-K1-G1-002_C12	231	lupus la	3.88815E-22	78.8%	C:cytosol; F:nucleic acid binding; C:ribonucleoprotein complex; C:mitochondrion; C:nucleus
Mado-00-K1-G1-002_D03	221	60s ribosomal protein	3.45263E-18	96.80%	F:structural constituent of ribosome; C:large ribosomal subunit; P:translation
Mado-00-K1-G1-002_D07	270	phosphoribosylanthranilate transferase	3.47299E-39	94.95%	F:transferase activity, transferring glycosyl groups
Mado-00-K1-G1-002_D08	342	plasma membrane intrinsic protein	1.52988E-50	98.35%	C:integral to membrane; F:water channel activity; P:transmembrane transport
Mado-00-K1-G1-002_D10	257	tocopherol cyclase	1.43507E-40	86.55%	P:response to oxidative stress; P:fatty acid metabolic process; P:chlorophyll metabolic process; P:vitamin E biosynthetic process; P:response to temperature stimulus; F:tocopherol cyclase activity; C:chloroplast inner membrane; C:plastoglobule; P:regulation of defense response; P:xanthophyll metabolic process; P:response to high light intensity; P:phloem loading
Mado-00-K1-G1-002_D11	225	conserved hypothetical protein [Ricinus communis]	6.06753E-7	60.00%	C:integral to membrane
Mado-00-K1-G1-002_D12	392	metallothionein-like protein	7.17367E-29	78.65%	F:metal ion binding
Mado-00-K1-G1-002_E02	277	nuclear transcription factor y subunit a-	5.1528E-35	79.20%	F:transcription factor activity; C:CCAAT-binding factor complex; P:regulation of transcription, DNA-dependent
Mado-00-K1-G1-002_E03	339	asparagine synthetase	7.88458E-44	79.05%	F:asparagine synthase (glutamine-hydrolyzing) activity; P:glutamine metabolic process; P:asparagine biosynthetic process; F:ATP binding
Mado-00-K1-G1-002_E04	353	ferredoxin	3.28588E-21	91.90%	F:metal ion binding; P:electron transport chain; F:electron carrier activity; F:2 iron, 2 sulfur cluster binding; F:protein binding; C:chloroplast; P:transport
Mado-00-K1-G1-002_E06	370	protein	2.99557E-30	70.20%	F:transferase activity
Mado-00-K1-G1-002_E08	315	14-3-3 protein	8.72316E-30	96.30%	P:defense response to bacterium; F:protein domain specific binding; P:response to cadmium ion; C:plasma membrane; F:protein phosphorylated amino acid binding; C:cell wall; C:nucleus; C:cytosol; P:brassinosteroid mediated signaling pathway
Mado-00-K1-G1-002_E09	441	auxin signaling f-box 2	9.85708E-58	74.45%	P:response to molecule of bacterial origin; P:stamen development; F:protein binding; P:pollen maturation; F:ubiquitin-protein ligase activity; P:auxin mediated signaling pathway; F:inositol hexakisphosphate binding; F:auxin binding
Mado-00-K1-G1-002_E10	178	glycosyltransferase ugt95a1	2.16767E-17	78.08%	F:anthocyanidin 3-O-glucosyltransferase activity; F:metal ion binding; P:metabolic process
Mado-00-K1-G1-002_E12	324	leucine-rich repeat family protein	2.27518E-22	97.80%	P:auxin biosynthetic process; P:protein amino acid phosphorylation; C:plasma membrane; F:protein binding; F:ATP binding; C:integral to membrane; F:protein serine/threonine kinase activity; C:mitochondrion
Mado-00-K1-G1-002_F01	427	protein	2.47532E-69	90.65%	F:alpha,alpha-trehalose-phosphate synthase (UDP-forming) activity; F:protein binding; F:glucosylglycerol-phosphate synthase activity; F:trehalose-phosphatase activity; P:trehalose biosynthetic process; C:mitochondrion

Mado-00-K1-G1-002_F02	371	mei2-like protein	1.68919E-49	83.75%	P:translation; C:cell wall; F:RNA binding; P:meristem development; F:structural constituent of ribosome; P:positive regulation of meiosis; C:cytosolic small ribosomal subunit; F:protein binding; F:nucleotide binding; C:chloroplast; C:vacuole; C:plasma membrane
Mado-00-K1-G1-002_F04	252	like protein	1.96447E-5	77.00%	F:ATP binding; C:cytoplasm; P:protein folding
Mado-00-K1-G1-002_F06	288	atp synthase d mitochondrial	1.30041E-25	90.75%	F:copper ion binding; F:hydrogen ion transmembrane transporter activity; F:zinc ion binding; C:mitochondrial proton-transporting ATP synthase complex, coupling factor F(o); C:nucleolus; P:response to salt stress; P:ATP synthesis coupled proton transport; C:thylakoid; F:protein binding; C:chloroplast; F:hydrolase activity; C:cytosolic ribosome; C:plasma membrane
Mado-00-K1-G1-002_F07	182	beta-alanine synthase	2.84367E-12	90.40%	F:N-carbamoylputrescine amidase activity; F:beta-ureidopropionase activity; P:putrescine biosynthetic process
Mado-00-K1-G1-002_F08	285	60s ribosomal protein l23	2.70247E-47	99.60%	C:nucleolus; F:structural constituent of ribosome; P:embryonic development ending in seed dormancy; C:cytosolic large ribosomal subunit; P:translation; C:mitochondrion
Mado-00-K1-G1-002_F10	542	cell division cycle protein 23	2.57635E-87	87.80%	P:regulation of mitotic metaphase/anaphase transition; F:binding; C:anaphase-promoting complex; P:cell division
Mado-00-K1-G1-002_F11	257	glutathione s-transferase	1.0988E-40	87.85%	P:toxin catabolic process; P:defense response to bacterium; F:glutathione transferase activity; C:vacuole; F:copper ion binding; F:nucleic acid binding; P:response to cadmium ion; C:plasma membrane; P:response to zinc ion; C:chloroplast stroma; F:glutathione peroxidase activity; F:zinc ion binding; C:apoplast; F:glutathione binding
Mado-00-K1-G1-002_F12	572	gtp-binding family protein	5.22997E-31	76.00%	C:intracellular; F:GTP binding
Mado-00-K1-G1-002_G01	180	dna binding	2.32088E-22	83.79%	F:DNA binding; F:nuclease activity; P:nucleotide-excision repair; C:chloroplast; C:mitochondrion
Mado-00-K1-G1-002_G05	321	protein	5.04069E-54	94.60%	P:protein amino acid glycosylation; C:membrane; F:dolichyl-diphosphooligosaccharide-protein glycotransferase activity; C:endoplasmic reticulum; C:plastid
Mado-00-K1-G1-002_G02	273	protein	5.74407E-18	66.60%	F:protein binding
Mado-00-K1-G1-002_G07	139	sorbitol transporter	1.18982E-15	91.20%	F:substrate-specific transmembrane transporter activity; C:integral to membrane; P:oxidation reduction; P:transmembrane transport; F:2-alkenal reductase activity; P:carbohydrate transport
Mado-00-K1-G1-002_G08	134	cellular retinaldehyde-binding triple c-terminal	3.26792E-5	75.57%	
Mado-00-K1-G1-002_G09	237	dna-damage-repair toleration protein drt102	4.58283E-7	76.75%	P:carbohydrate metabolic process
Mado-00-K1-G1-002_G10	420	chloroplast alpha-glucan water	1.25338E-28	76.30%	F:carbohydrate kinase activity; F:binding; F:phosphoglucan, water dikinase activity; P:starch catabolic process; C:chloroplast stroma; P:protein amino acid autophosphorylation
Mado-00-K1-G1-002_G12	413	glyceraldehyde 3-phosphate	7.12102E-69	97.90%	C:cytoplasm; F:NAD or NADH binding; P:oxidation reduction; P:glycolysis; F:glyceraldehyde-3-phosphate dehydrogenase (phosphorylating) activity
Mado-00-K1-G1-002_H01	260	probable prefoldin subunit 2	3.55519E-7	84.25%	P:protein folding; C:prefoldin complex; F:unfolded protein binding
Mado-00-K1-G1-002_H03	298	af412084_1at3g11590 f24k9_26	3.16092E-32	67.64%	C:plasma membrane
Mado-00-K1-G1-002_H04	225	phosphoenolpyruvate carboxylase	3.08741E-35	95.00%	P:oxidation reduction; P:tricarboxylic acid cycle; F:phosphoenolpyruvate carboxylase activity; F:2-alkenal reductase activity
Mado-00-K1-G1-002_H06	531	alpha beta fold family protein	1.48186E-31	79.40%	F:acylglycerol lipase activity; F:nucleic acid binding; C:plastid
Mado-00-K1-G1-002_H09	313	membrane protein ch1-like	2.01851E-26	68.35%	C:mitochondrion; C:membrane
Mado-00-K1-G1-002_H12	223	60s ribosomal protein l30	4.6399E-23	92.15%	F:structural constituent of ribosome; C:cytosolic large ribosomal subunit; P:translation
Mado-00-K1-G1-003_A01	243	60s ribosomal protein l13a	5.33909E-32	96.45%	F:structural constituent of ribosome; C:large ribosomal subunit; P:translation
Mado-00-K1-G1-003_A02	478	aluminum-induced protein	2.08539E-44	90.85%	P:response to auxin stimulus; P:response to aluminum ion
Mado-00-K1-G1-003_A03	162	peroxisomal ascorbate peroxidase	1.47407E-21	95.50%	F:heme binding; P:oxidation reduction; F:L-ascorbate peroxidase activity; P:response to oxidative stress
Mado-00-K1-G1-003_A05	502	multiubiquitin chain binding protein mbp1	3.98995E-75	93.60%	P:response to misfolded protein; P:stamen formation; P:response to sucrose stimulus; P:proteasomal ubiquitin-dependent protein catabolic process; P:response to DNA damage stimulus; C:cytoplasmic membrane-bounded vesicle; F:peptide receptor activity; P:response to salt stress; P:proteasome core complex assembly; P:root hair elongation; C:cytosol; P:regulation of seed germination; P:leaf senescence; P:pollen development; C:proteasome regulatory particle, base subcomplex; P:response to heat; P:leaf development; P:response to abscisic acid stimulus; P:post-embryonic root development; P:response to cytokinin stimulus; C:nucleus; P:response to auxin stimulus
Mado-00-K1-G1-003_A06	120	protein	8.98862E-11	85.30%	F:transcription regulator activity; P:regulation of transcription; C:nucleus; C:cytosol; F:calmodulin binding
Mado-00-K1-G1-003_A07	180	unknown [Glycine max]	6.54056E-17	81.89%	F:molecular_function; P:biological_process; C:cellular_component
Mado-00-K1-G1-003_A11	468	zeaxanthin epoxidase	3.61393E-36	62.50%	F:monooxygenase activity; P:metabolic process; C:chloroplast; C:membrane
Mado-00-K1-G1-003_B01	379	ubiquitin carboxyl-terminal	6.82381E-27	84.45%	P:ubiquitin-dependent protein catabolic process; F:ubiquitin-specific protease activity; F:ubiquitin thiolesterase activity
Mado-00-K1-G1-003_B04	216	malate oxidoreductase	1.35656E-11	87.76%	F:malate dehydrogenase (decarboxylating) activity; F:cobalt ion binding; F:NAD or NADH binding; C:mitochondrial matrix; P:malate metabolic process; P:oxidation reduction; F:ATP binding; F:oxidoreductase activity, acting on NADH or NADPH, NAD or NADP as acceptor; C:chloroplast; F:zinc ion binding
Mado-00-K1-G1-003_B07	274	pectin methylesterase	4.09743E-32	84.85%	F:pectinesterase activity; P:cell wall modification; C:plant-type cell wall; C:cytoplasm; C:apoplast; F:aspartyl esterase activity; P:response to nematode; P:cellular cell wall organization; F:enzyme inhibitor activity
Mado-00-K1-G1-003_B08	330	dolichyl-di-phosphooligosaccharide-protein glycotransferase -like	4.54978E-23	86.75%	C:oligosaccharyltransferase complex; P:protein amino acid N-linked glycosylation via asparagine; P:cellulose biosynthetic process; C:plant-type cell wall; C:cytoplasmic membrane-bounded vesicle; P:unidimensional cell growth; P:plant-type cell wall organization; C:vacuole; F:dolichyl-diphosphooligosaccharide-protein glycotransferase activity; C:plasma membrane
Mado-00-K1-G1-003_B09	205	protein	9.80691E-26	88.40%	F:GTP binding; C:membrane
Mado-00-K1-G1-003_C02	321	xyloglucan endotransglycosylase	9.48431E-61	92.95%	F:hydrolase activity, hydrolyzing O-glycosyl compounds; P:cellular glucan metabolic process; C:apoplast; C:cell wall; F:xyloglucan:xyloglucosyl transferase activity
Mado-00-K1-G1-003_C07	229	glycosyl hydrolase family 38 protein	5.44396E-24	79.45%	C:plant-type cell wall; C:vacuole; F:alpha-mannosidase activity; C:cytoplasmic membrane-bounded vesicle; F:carbohydrate binding; C:plasma membrane; P:mannose metabolic process; F:zinc ion binding; C:apoplast
Mado-00-K1-G1-003_C08	360	60s ribosomal protein l21	1.60802E-28	92.00%	C:ribosome; F:structural constituent of ribosome; P:translation; C:mitochondrion
Mado-00-K1-G1-003_C09	254	at5g11580 f15n18_170	4.98406E-17	83.13%	F:chromatin binding; F:Ran GTPase binding
Mado-00-K1-G1-003_C10	351	tubulin beta	3.83674E-62	99.65%	P:microtubule-based movement; F:GTP binding; P:protein polymerization; F:structural molecule activity; F:GTPase activity; C:microtubule
Mado-00-K1-G1-003_C12	246	protein	1.60572E-20	72.75%	C:membrane; P:response to hormone stimulus; P:response to light stimulus
Mado-00-K1-G1-003_D08	279	myc transcription factor	4.08711E-16	89.75%	P:regulation of transcription factor activity; P:jasmonic acid mediated signaling pathway; P:response to desiccation; P:response to wounding; P:oxidation reduction; F:2-alkenal reductase activity; P:positive regulation of flavonoid biosynthetic process; P:response to chitin; P:response to abscisic acid stimulus; F:transcription activator activity; F:protein binding; F:transcription factor activity; P:regulation of transcription from RNA polymerase II promoter in response to oxidative stress; C:nucleus; P:positive regulation of transcription
Mado-00-K1-G1-003_D02	208	glycosyl hydrolase family 17 protein	1.41798E-8	84.56%	P:carbohydrate metabolic process; F:cation binding; C:anchored to plasma membrane; C:cytoplasmic membrane-bounded vesicle; F:glucan endo-1,3-beta-D-glucosidase activity
Mado-00-K1-G1-003_D04	561	alanine aminotransferase	9.54042E-83	95.50%	P:response to oxidative stress; C:peroxisome; F:alanine-glyoxylate transaminase activity; F:gamma-glutamyltransferase activity; C:plant-type cell wall; F:obs-aminocyclopropane-1-carboxylate synthase activity; F:L-alanine:2-oxoglutarate aminotransferase activity; F:glutathione gamma-glutamylcysteinyltransferase activity; C:chloroplast stroma; P:glutathione catabolic process; P:photorespiration; C:vacuole; F:pyridoxal phosphate binding; C:membrane; F:glycine:2-oxoglutarate aminotransferase activity; C:apoplast

Mado-00-K1-G1-003_D10	187	60s ribosomal protein l35a	1.59687E-23	91.05%	F:structural constituent of ribosome; P:ribosome biogenesis; C:cytosolic large ribosomal subunit; C:membrane; P:translation
Mado-00-K1-G1-003_E01	318	flavonoid 3-	1.1773E-18	81.85%	F:heme binding; F:flavonoid 3',5'-hydroxylase activity; P:oxidation reduction; F:oxidoreductase activity, acting on single donors with incorporation of molecular oxygen, incorporation of two atoms of oxygen; F:electron carrier activity; F:flavonoid 3'-monooxygenase activity
Mado-00-K1-G1-003_E02	188	sorbitol transporter	1.65634E-12	78.25%	F:substrate-specific transmembrane transporter activity; C:integral to membrane; P:transmembrane transport
Mado-00-K1-G1-003_E07	306	homocysteine s-methyltransferase	5.88216E-39	81.90%	P:S-methylmethionine cycle; F:homocysteine S-methyltransferase activity; P:methionine biosynthetic process
Mado-00-K1-G1-003_D06	481	sorting nexin 1	6.02602E-52	89.20%	P:positive gravitropism; P:root development; P:intracellular signaling pathway; C:retromer complex; C:multivesicular body; P:protein targeting to vacuole; P:endosome to lysosome transport; P:Golgi to vacuole transport; C:microsome; F:protein binding; P:auxin homeostasis; P:cell communication; F:phosphoinositide binding
Mado-00-K1-G1-003_E03	419	starch phosphorylase	2.60023E-58	92.10%	F:identical protein binding; P:response to water deprivation; F:phosphorylase activity; P:carbohydrate metabolic process; F:pyridoxal phosphate binding; C:amyloplast; C:chloroplast stroma; P:response to temperature stimulus
Mado-00-K1-G1-003_E06	147	mgatp-energized glutathione s-conjugate	9.40163E-16	90.60%	P:transmembrane transport; P:auxin biosynthetic process; C:integral to membrane; F:ATPase activity, coupled to transmembrane movement of substances; C:plant-type vacuole; P:oxidation reduction; F:2-alkenal reductase activity; C:vacuolar membrane; F:ATP binding; C:plasma membrane
Mado-00-K1-G1-003_E08	490	protein	2.7381E-68	78.20%	C:plasma membrane; F:protein binding; F:protein transporter activity; P:vesicle-mediated transport; P:intracellular protein transport; C:clathrin adaptor complex
Mado-00-K1-G1-003_E09	445	60s ribosomal protein	2.06073E-31	86.40%	F:structural constituent of ribosome; C:plasma membrane; P:ribosome biogenesis; C:cytosolic large ribosomal subunit; P:translation; C:mitochondrion
Mado-00-K1-G1-003_F03	442	protein	5.81911E-34	86.60%	F:polyamine oxidase activity; C:peroxisome; P:polyamine catabolic process; F:amine oxidase activity; P:oxidation reduction; F:methyltransferase activity
Mado-00-K1-G1-003_E12	355	protein	3.14828E-40	93.75%	F:structural constituent of ribosome; C:cytosolic small ribosomal subunit; C:membrane; P:translation
Mado-00-K1-G1-003_F02	206	PREDICTED: hypothetical protein [Vitis vinifera]	2.42114E-16	79.00%	C:endomembrane system; C:membrane; C:cytoplasmic membrane-bounded vesicle
Mado-00-K1-G1-003_F06	387	starch branching enzyme i	4.49073E-47	76.00%	F:hydrolase activity, hydrolyzing O-glycosyl compounds; P:carbohydrate metabolic process; F:cation binding; F:obs,4-alpha-glucan branching enzyme activity; C:plastid
Mado-00-K1-G1-003_F07	272	auxin efflux carrier family protein	2.5936E-10	58.50%	P:auxin polar transport; C:integral to membrane; P:transmembrane transport; F:auxin:hydrogen symporter activity
Mado-00-K1-G1-003_F11	277	protein	2.30763E-43	97.25%	F:translation elongation factor activity; P:response to cold; F:GTP binding; F:copper ion binding; C:plasma membrane; C:chloroplast; C:cytosol; F:GTPase activity
Mado-00-K1-G1-003_F12	184	protein	4.52532E-10	65.00%	P:multidimensional cell growth; P:microtubule bundle formation; C:cortical microtubule; P:establishment or maintenance of cell polarity; P:circumnutation; P:root morphogenesis; P:trichome branching
Mado-00-K1-G1-003_G02	250	cinnamoyl- reductase	1.35775E-11	79.45%	F:cinnamoyl-CoA reductase activity; P:response to cadmium ion; F:dihydrokaempferol 4-reductase activity; P:oxidation reduction; P:lignin biosynthetic process; F:coenzyme binding
Mado-00-K1-G1-003_G04	173	protein	5.92754E-23	97.20%	F:peptidase activity; P:ubiquitin-dependent protein catabolic process; P:blue light signaling pathway; P:auxin biosynthetic process; F:signal transducer activity; C:proteasome regulatory particle, base subcomplex; P:phototropism; F:ATP binding; F:microtubule-severing ATPase activity; F:protein binding; C:cytoplasm; C:nucleus; C:plasma membrane
Mado-00-K1-G1-003_G07	222	chromatin remodeling complex subunit	1.54103E-26	78.80%	P:response to water deprivation; P:response to salt stress; F:ATP binding; F:helicase activity; F:DNA binding; F:transferase activity; P:response to heat
Mado-00-K1-G1-003_G08	306	unnamed protein product [Vitis vinifera]	1.65E-49	91.10%	C:anchored to membrane; C:cytoplasmic membrane-bounded vesicle; C:plasma membrane
Mado-00-K1-G1-003_G11	316	aspartokinase-homoserine dehydrogenase	1.81047E-43	89.95%	P:methionine biosynthetic process; F:homoserine dehydrogenase activity; P:oxidation reduction; F:aspartate kinase activity; F:ATP binding; F:aminoacyl-tRNA ligase activity; P:tRNA aminoacylation for protein translation; F:amino acid binding; C:chloroplast; F:NADP or NADPH binding
Mado-00-K1-G1-003_G12	162	wrky12	4.59293E-15	78.70%	F:sequence-specific DNA binding; P:regulation of transcription; F:transcription factor activity; C:nucleus
Mado-00-K1-G1-003_H07	343	PREDICTED: hypothetical protein [Vitis vinifera]	4.63851E-23	79.25%	C:cytoplasmic membrane-bounded vesicle; C:mitochondrion; C:plastid
Mado-00-K1-G1-003_H08	154	protein	2.91102E-9	63.17%	F:metal ion binding; F:ubiquitin-protein ligase activity; F:protein binding; P:plant-type hypersensitive response; C:plasma membrane
Mado-00-K1-G1-003_H10	513	autophagy protein 9	2.43546E-54	56.80%	P:autophagy
Mado-00-K1-G1-003_H11	334	bzip domain class transcription factor	4.85381E-17	89.55%	F:signal transducer activity; P:phototropism; C:nucleus; F:protein binding
Mado-00-K1-G1-004B_A05	422	diacylglycerol kinase	1.87292E-24	75.25%	P:activation of protein kinase C activity by G-protein coupled receptor protein signaling pathway; F:diacylglycerol kinase activity
Mado-00-K1-G1-004B_A07	173	ac083943_21 oxidoreductase	3.04429E-19	85.70%	P:oxidation reduction; F:oxidoreductase activity; F:binding; C:plastid
Mado-00-K1-G1-004B_A08	287	protein	5.12034E-30	98.80%	F:RNA binding; P:translational initiation; F:translation initiation factor activity
Mado-00-K1-G1-004B_A09	497	atp binding atpase nucleoside-triphosphatase nucleotide binding	1.59415E-60	75.40%	P:auxin biosynthetic process; F:ATPase activity; F:ATP binding; C:mitochondrion
Mado-00-K1-G1-004B_A10	438	60s ribosomal protein	2.27745E-67	96.60%	C:ribosome; F:structural constituent of ribosome; P:translation
Mado-00-K1-G1-004B_A12	260	tryptophanyl-trna synthetase	7.58284E-42	92.30%	P:auxin biosynthetic process; F:tryptophan-tRNA ligase activity; P:tryptophanyl-tRNA aminoacylation; F:ATP binding; C:cytosol
Mado-00-K1-G1-004B_B01	219	protein translocase protein transporter	1.1839E-26	84.70%	F:P-P-bond-hydrolysis-driven protein transmembrane transporter activity; C:plastid outer membrane; C:respiratory chain complex I; C:mitochondrial inner membrane presequence translocase complex; C:integral to membrane; C:chloroplast; P:protein transport
Mado-00-K1-G1-004B_B03	212	pyrophosphate-energized vacuolar membrane proton	1.1881E-31	98.20%	F:hydrogen-translocating pyrophosphatase activity; C:vacuolar membrane; P:oxidation reduction; P:proton transport; F:inorganic diphosphatase activity; C:integral to membrane; F:2-alkenal reductase activity
Mado-00-K1-G1-004B_B04	293	a chain mechanism of auxin perception by the tir1 ubiquitin ligase	6.44547E-17	94.00%	P:ubiquitin-dependent protein catabolic process; F:protein kinase activity; P:embryonic development ending in seed dormancy; P:response to salt stress; C:SCF ubiquitin ligase complex; F:ubiquitin-protein ligase activity; F:protein binding; P:mitosis; P:negative regulation of DNA recombination; C:spindle; C:phragmoplast; P:male meiosis; C:nucleus; C:plasma membrane; P:protein amino acid phosphorylation
Mado-00-K1-G1-004B_B06	213	dehydrin-like protein	5.87678E-31	74.50%	P:response to water; P:response to stress
Mado-00-K1-G1-004B_B02	231	protein	3.88815E-22	77.55%	C:mitochondrion
Mado-00-K1-G1-004B_B08	223	cp protein	5.68506E-13	86.95%	F:molecular_function; P:biological_process; C:cellular_component
Mado-00-K1-G1-004B_B10	251	carbon-nitrogen hydrolase family protein	3.32879E-26	93.25%	P:nitrogen compound metabolic process; F:nitrilase activity; F:zinc ion binding; C:chloroplast
Mado-00-K1-G1-004B_B11	340	peroxisomal biogenesis factor 11 family protein	7.60149E-55	94.10%	C:integral to peroxisomal membrane; P:peroxisome fission; C:mitochondrion
Mado-00-K1-G1-004B_C03	147	coat protein	1.03706E-22	100.00%	F:structural molecule activity; C:viral capsid
Mado-00-K1-G1-004B_C07	337	adenine nucleotide translocator	3.09802E-56	98.00%	F:binding; C:mitochondrial inner membrane; P:transmembrane transport; C:integral to membrane; F:transporter activity
Mado-00-K1-G1-004B_C08	173	26s protease regulatory	1.46E-21	93.05%	C:cytoplasm; P:auxin biosynthetic process; F:peptidase activity; C:proteasome regulatory particle, base subcomplex; P:ubiquitin-dependent protein catabolic process; F:ATP binding; F:microtubule-severing ATPase activity; C:nucleus
Mado-00-K1-G1-004B_C09	165	protein	1.46938E-21	98.40%	C:extrinsic to membrane; F:calcium ion binding; P:photosystem II stabilization; C:chloroplast thylakoid membrane; C:oxygen evolving complex

	Mado-00-K1-G1-004B_C10	194	amp deaminase	1.70289E-9	85.50%	F:AMP deaminase activity; C:cytosol; P:embryonic development ending in seed dormancy; P:purine ribonucleoside monophosphate biosynthetic process; C:microsome; F:protein binding; C:nucleus
	Mado-00-K1-G1-004B_D02	189	omega-3 fatty acid desaturase	8.15567E-28	92.35%	C:endoplasmic reticulum membrane; F:delta12-fatty acid dehydrogenase activity; F:omega-3 fatty acid desaturase activity; P:oxidation reduction; P:unsaturated fatty acid biosynthetic process; C:integral to membrane
	Mado-00-K1-G1-004B_D06	198	binding protein	4.15724E-16	76.13%	F:binding
	Mado-00-K1-G1-004B_D07	400	af380625_1 at3g02420 f16b3_5	9.54389E-37	72.20%	C:integral to membrane
	Mado-00-K1-G1-004B_D08	453	transcription factor	1.36743E-19	82.05%	P:chromatin assembly or disassembly; C:chromatin; C:nucleus; F:transcription factor activity; F:structural constituent of chromatin; F:chromatin binding
	Mado-00-K1-G1-004B_D09	238	protein	5.93009E-39	94.10%	P:auxin biosynthetic process; C:nucleolus; C:chloroplast envelope; F:RNA helicase activity; F:ATP-dependent helicase activity; F:nucleic acid binding; F:ATP binding; C:membrane
	Mado-00-K1-G1-004B_E04	461	60s ribosomal protein l24	8.74751E-29	94.95%	P:gynoecium development; P:translation; F:structural constituent of ribosome; P:ribosome biogenesis; P:auxin mediated signaling pathway; C:cytosolic large ribosomal subunit; C:plasma membrane
	Mado-00-K1-G1-004B_E05	307	predicted protein [Populus trichocarpa]	1.32262E-6	67.57%	
	Mado-00-K1-G1-004B_E06	163	60s ribosomal protein l34	7.33273E-13	93.00%	C:ribosome; F:structural constituent of ribosome; P:translation
	Mado-00-K1-G1-004B_E07	147	protein	2.09446E-15	80.59%	C:endomembrane system; C:cytoplasmic membrane-bounded vesicle; C:membrane
	Mado-00-K1-G1-004B_E12	461	protein	4.28995E-74	95.70%	F:ATP binding; P:oxidation reduction; P:response to stress; P:auxin biosynthetic process; F:2-alkenal reductase activity
	Mado-00-K1-G1-004B_F03	353	purple acid phosphatase	8.29801E-49	85.50%	P:seed germination; F:metal ion binding; F:protein serine/threonine phosphatase activity; P:pollen germination; F:acid phosphatase activity
	Mado-00-K1-G1-004B_F05	522	protein	3.37131E-86	78.05%	F:myosin heavy chain kinase activity; P:multicellular organismal development; F:nucleotide binding; C:heterotrimeric G-protein complex
	Mado-00-K1-G1-004B_F06	116	atp-binding cassette	8.17953E-12	90.20%	P:auxin biosynthetic process; F:xenobiotic-transporting ATPase activity; F:ATP binding; F:polyamine-transporting ATPase activity; F:phosphonate transmembrane-transporting ATPase activity; F:heme-transporting ATPase activity; C:membrane
	Mado-00-K1-G1-004B_F08	335	protein	2.48707E-21	84.79%	C:membrane
	Mado-00-K1-G1-004B_F11	133	ferredoxin-nadp+ reductase	8.85822E-19	99.85%	P:transport; P:electron transport chain; C:thylakoid lumen; F:poly(U) RNA binding; F:NADPH dehydrogenase activity; F:electron transporter, transferring electrons within the noncyclic electron transport pathway of photosynthesis activity; P:defense response to bacterium; C:chloroplast envelope; F:ferredoxin-NADP+ reductase activity; F:protein binding; C:chloroplast thylakoid membrane; C:chloroplast stroma; P:photosynthesis; F:FAD binding; F:NADP or NADPH binding; F:electron transporter, transferring electrons within the cyclic electron transport pathway of photosynthesis activity; C:apoplast
	Mado-00-K1-G1-004B_F10	230	glutaredoxin [Populus tremula x Populus tremuloides]	3.66858E-4	82.00%	P:cell redox homeostasis; F:electron carrier activity; F:protein disulfide oxidoreductase activity
	Mado-00-K1-G1-004B_F09	294	nodulation receptor kinase	1.7408E-30	75.65%	P:auxin biosynthetic process; F:receptor activity; P:protein amino acid phosphorylation; C:cytoplasmic membrane-bounded vesicle; C:plasma membrane; F:ATP binding; F:MAP kinase kinase kinase activity
	Mado-00-K1-G1-004B_G02	306	hva22d	1.19073E-23	89.55%	P:hyperosmotic salinity response; P:pollen development; P:response to water deprivation; C:endomembrane system; P:response to cold; P:negative regulation of autophagy; P:response to abscisic acid stimulus; P:flower development; C:integral to membrane
	Mado-00-K1-G1-004B_G03	337	stress-induced protein sti1-like protein	2.54612E-42	92.30%	P:response to cadmium ion; P:response to high light intensity; C:cytosol; P:response to hydrogen peroxide; F:binding; C:nucleus; C:plasma membrane; P:response to heat
	Mado-00-K1-G1-004B_G04	342	protein	9.32463E-32	64.05%	F:DNA-directed RNA polymerase activity; F:DNA binding; P:transcription; F:ribonucleoside binding; C:nucleus; F:zinc ion binding
	Mado-00-K1-G1-004B_G05	240	ribosomal protein s12	5.35754E-32	100.00%	F:structural constituent of ribosome; C:small ribosomal subunit; C:chloroplast; P:translation; F:rRNA binding
	Mado-00-K1-G1-004B_G06	452	asparagine synthetase	4.7951E-81	96.80%	F:asparagine synthase (glutamine-hydrolyzing) activity; P:glutamine metabolic process; P:asparagine biosynthetic process; F:ATP binding
	Mado-00-K1-G1-004B_G07	463	iron-sulfur assembly protein	1.68202E-57	88.40%	P:iron-sulfur cluster assembly; F:structural molecule activity; F:iron-sulfur cluster binding; C:chloroplast stroma
	Mado-00-K1-G1-004B_G08	179	orf16-lacz fusion protein	2.08985E-28	70.65%	C:mitochondrion
	Mado-00-K1-G1-004B_G09	260	membrane protein cov	4.91505E-41	94.95%	F:molecular_function; P:biological_process; C:cellular_component
	Mado-00-K1-G1-004B_G10	316	unnamed protein product [Vitis vinifera]	1.30956E-9	76.14%	F:molecular_function; P:biological_process; C:cellular_component
	Mado-00-K1-G1-004B_G11	402	aldo keto reductase family protein	1.71616E-70	88.75%	F:steroid dehydrogenase activity; C:cytosol; P:response to water deprivation; P:response to salt stress; P:oxidation reduction; C:nucleus; P:response to cold; F:aldehyde reductase activity
	Mado-00-K1-G1-004B_H04	297	cystathionine gamma-synthase	4.10919E-48	91.05%	F:pyridoxal phosphate binding; F:cystathionine gamma-synthase activity; P:methionine biosynthetic process; F:lyase activity; C:chloroplast
	Mado-00-K1-G1-004B_H05	186	pear beta-galactosidase3	5.30361E-27	79.25%	F:sugar binding; P:carbohydrate metabolic process; C:plant-type cell wall; C:vacuole; F:cation binding; C:cytoplasmic membrane-bounded vesicle; C:plasma membrane; F:beta-galactosidase activity
	Mado-00-K1-G1-004B_H08	267	sugar binding protein	2.25913E-38	77.45%	F:sugar binding; F:transferase activity, transferring glycosyl groups; P:GPI anchor biosynthetic process; C:intrinsic to endoplasmic reticulum membrane
	Mado-00-K1-G1-004B_H12	153	predicted protein [Populus trichocarpa]	1.10619E-8	88.50%	C:mitochondrion
	Mado-00-K1-G1-004B_H09	271	cytochrome p450	7.03037E-24	74.75%	F:oxidoreductase activity; F:iron ion binding
	Mado-00-K1-G1-004B_H11	439	unknown [Glycine max]	2.08389E-28	70.35%	
K2-G2	Mado-00-K2-G2-Contig1	229	hypothetical protein ARALYDRAFT_470811 [Arabidopsis lyrata subsp. lyrata]	8.83085E-14	100.00%	C:nucleosome; F:DNA binding; P:nucleosome assembly; C:nucleus
	Mado-00-K2-G2-Contig2	404	chloroplast light-harvesting chlorophyll a b-binding protein	1.20806E-51	96.05%	F:metal ion binding; P:photosynthesis, light harvesting; C:photosystem II; P:protein-chromophore linkage; F:chlorophyll binding; C:integral to membrane; C:photosystem I; C:chloroplast thylakoid membrane
	Mado-00-K2-G2-Contig4	393	tubulin alpha	3.68111E-60	99.00%	P:microtubule-based movement; F:GTP binding; P:protein polymerization; F:structural molecule activity; F:GTPase activity; C:microtubule
	Mado-00-K2-G2-Contig5	383	protein	1.37866E-51	79.50%	P:response to abscisic acid stimulus; C:membrane; C:cytoplasmic membrane-bounded vesicle; P:response to water deprivation; P:response to cold; P:hyperosmotic salinity response
	Mado-00-K2-G2-Contig6	268	senescence-associated protein	9.93368E-42	93.50%	F:transcription factor binding; F:transcription factor activity; P:rRNA transcription; P:cell proliferation
	Mado-00-K2-G2-Contig7	171	protein	6.23232E-12	68.35%	C:mitochondrion; F:peptidase activity
	Mado-00-K2-G2-Contig8	166	stem 28 kda glycoprotein	2.63525E-10	80.55%	F:acid phosphatase activity
	Mado-00-K2-G2-Contig9	466	lipid binding	4.36755E-21	69.70%	P:lipid transport
	Mado-00-K2-G2-Contig10	460	metallothionein-like protein	5.24858E-26	87.15%	F:metal ion binding
	Mado-00-K2-G2-Contig11	401	histone 2	4.96909E-33	92.40%	C:nucleosome; F:DNA binding; P:nucleosome assembly; C:nucleus
	Mado-00-K2-G2-Contig12	331	ferrodoxin precursor	7.25064E-21	92.05%	F:metal ion binding; F:electron carrier activity; F:protein binding; P:transport; C:chloroplast stroma; F:2 iron, 2 sulfur cluster binding; P:electron transport chain
	Mado-00-K2-G2-Contig14	309	proline-rich protein	2.04658E-31	73.10%	F:structural constituent of cell wall; P:response to jasmonic acid stimulus; C:plasma membrane
	Mado-00-K2-G2-Contig15	277	expansin 1	1.79112E-43	99.10%	C:extracellular region; P:plant-type cell wall organization; C:membrane
	Mado-00-K2-G2-Contig16	246	stem 28 kda glycoprotein	4.90091E-17	91.25%	C:cytoplasmic membrane-bounded vesicle; C:mitochondrion; F:acid phosphatase activity
	Mado-00-K2-G2-Contig18	420	auxin efflux carrier	2.36872E-67	96.10%	P:transmembrane transport; C:integral to membrane
	Mado-00-K2-G2-Contig19	402	sah7 protein	9.03956E-27	75.35%	C:extracellular space
	Mado-00-K2-G2-Contig20	400	40s ribosomal protein s28	1.55187E-18	92.75%	C:ribosome; F:structural constituent of ribosome; P:translation
	Mado-00-K2-G2-Contig21	352	histone h2	3.11776E-19	98.60%	C:nucleosome; F:DNA binding; P:nucleosome assembly; C:nucleus
	Mado-00-K2-G2-Contig22	333	predicted protein [Populus trichocarpa]	1.87412E-8	78.53%	C:endomembrane system; C:cytoplasmic membrane-bounded vesicle

Mado-00-K2-G2-Contig23	321	soul heme-binding family protein	9.35572E-40	75.20%	C:cytoplasmic membrane-bounded vesicle; C:vacuole; F:binding; C:plasma membrane
Mado-00-K2-G2-Contig24	319	cold acclimation protein cor413-pm1	8.22677E-36	88.60%	C:membrane
Mado-00-K2-G2-Contig25	317	gdsl-motif lipase hydrolase family protein	1.18962E-42	89.00%	C:endomembrane system; P:lipid metabolic process; F:carboxylesterase activity
Mado-00-K2-G2-Contig27	288	ribulose bisphosphate carboxylase	5.91735E-26	89.15%	P:reductive pentose-phosphate cycle; F:ribulose-bisphosphate carboxylase activity; F:monooxygenase activity; P:photorespiration; P:oxidation reduction; C:chloroplast
Mado-00-K2-G2-Contig28	288	endo- -beta-glucanase precursor	6.09516E-39	86.90%	P:carbohydrate metabolic process; P:pattern specification process; F:cellulase activity; P:response to nematode
Mado-00-K2-G2-Contig29	288	anthocyanidin reductase	2.16284E-44	92.50%	P:oxidation reduction; P:cellular metabolic process; F:coenzyme binding; F:anthocyanidin reductase activity
Mado-00-K2-G2-Contig30	278	hva22-like protein a	5.61974E-13	89.45%	P:response to abscisic acid stimulus; C:membrane; C:cytoplasmic membrane-bounded vesicle; P:response to water deprivation; P:hyperosmotic salinity response
Mado-00-K2-G2-Contig31	263	protein	6.96021E-27	77.70%	C:plastid
Mado-00-K2-G2-Contig32	260	at1g29980 t1p2_9	1.08008E-19	74.80%	C:anchored to membrane; C:cytoplasmic membrane-bounded vesicle; C:plasma membrane
Mado-00-K2-G2-Contig33	252	mitochondrial atp synthase g subunit family protein	1.73244E-25	95.55%	F:hydrogen ion transmembrane transporter activity; C:mitochondrial proton-transporting ATP synthase complex, coupling factor F(o); P:ATP synthesis coupled proton transport
Mado-00-K2-G2-Contig34	238	40s ribosomal protein s11	9.00354E-27	94.10%	F:structural constituent of ribosome; C:cell wall; P:embryonic development ending in seed dormancy; C:cytosolic small ribosomal subunit; C:membrane; P:translation; F:rRNA binding
Mado-00-K2-G2-Contig35	231	acid phosphatase	4.49907E-26	78.70%	C:cytoplasmic membrane-bounded vesicle; C:mitochondrion; F:acid phosphatase activity
Mado-00-K2-G2-Contig37	229	expansin 1	1.49587E-37	96.50%	C:extracellular region; P:plant-type cell wall organization; C:membrane
Mado-00-K2-G2-Contig41	206	nucleolar protein	4.46235E-26	92.30%	C:nucleolus; C:membrane; F:DNA binding
Mado-00-K2-G2-Contig43	183	leucine rich repeat protein	7.28967E-24	87.55%	P:defense response; F:kinase activity; F:protein binding; C:cell wall; P:oxidation reduction; P:signal transduction; C:chloroplast; C:membrane; C:apoplast; F:2-alkenal reductase activity
Mado-00-K2-G2-Contig45	180	import inner membrane translocase subunit mitochondrial	1.67698E-17	83.89%	C:mitochondrion; C:chloroplast
Mado-00-K2-G2-001_A01	410	zw18 expressed	9.23439E-40	88.10%	C:mitochondrion
Mado-00-K2-G2-001_A06	279	diphosphonucleotide phosphatase	5.2411E-11	86.85%	F:metal ion binding; F:protein serine/threonine phosphatase activity; F:acid phosphatase activity; C:chloroplast
Mado-00-K2-G2-001_A10	213	cell elongation protein	6.36471E-33	93.55%	P:brassinosteroid biosynthetic process; F:oxidoreductase activity; C:plasma membrane; P:unidimensional cell growth; C:integral to membrane; F:FAD binding; P:response to light stimulus; F:calmodulin binding
Mado-00-K2-G2-001_A12	299	protein	6.68134E-30	82.40%	F:hydrolase activity
Mado-00-K2-G2-001_B01	181	gpi-anchored protein	6.35776E-25	86.60%	C:anchored to membrane; C:cytoplasmic membrane-bounded vesicle
Mado-00-K2-G2-001_B02	370	60s ribosomal protein l7a	5.00555E-33	93.70%	C:ribosome; F:structural constituent of ribosome; F:protein binding; P:ribosome biogenesis; F:zinc ion binding; P:translation
Mado-00-K2-G2-001_B07	334	sinapyl alcohol dehydrogenase	7.10516E-16	79.35%	F:hydrolase activity, hydrolyzing O-glycosyl compounds; F:xyloglucan:xyloglucosyl transferase activity; F:binding; C:cell wall; P:cellular glucan metabolic process; C:apoplast
Mado-00-K2-G2-001_B08	358	seryl-trna synthetase	5.84771E-10	87.60%	F:serine-tRNA ligase activity; C:mitochondrion; P:seryl-tRNA aminoacylation; P:chloroplast organization; P:ovule development; F:ATP binding; P:mitochondrion organization; C:chloroplast
Mado-00-K2-G2-001_B09	357	60s ribosomal protein l34	2.13738E-28	97.05%	C:nucleolus; F:structural constituent of ribosome; P:ribosome biogenesis; C:cytosolic large ribosomal subunit; C:chloroplast; C:membrane; P:translation
Mado-00-K2-G2-001_C01	433	phenylalanine ammonia-lyase	5.72279E-74	94.70%	C:cytoplasm; P:phenylpropanoid metabolic process; P:biosynthetic process; F:ammonia-lyase activity; P:L-phenylalanine catabolic process
Mado-00-K2-G2-001_C02	372	sec14 cytosolic factor family protein phosphoglyceride transfer family protein	5.15942E-30	82.65%	C:cytosol; C:nucleus; P:transport; C:plasma membrane; F:transporter activity
Mado-00-K2-G2-001_C09	290	universal stress protein family protein	1.78167E-22	94.50%	P:response to stress; C:vacuole
Mado-00-K2-G2-001_C10	162	sec14 cytosolic factor family protein	2.30524E-22	86.89%	C:plastid; P:transport; C:plasma membrane; F:transporter activity
Mado-00-K2-G2-001_C11	179	zinc finger	2.05662E-15	75.25%	F:lipase activity; P:lipid metabolic process
Mado-00-K2-G2-001_D02	306	ubiquitin-like protein smt3	4.14863E-24	96.50%	C:cytoplasm; F:protein tag; P:protein sumoylation; F:protein binding; C:nucleus; P:response to heat
Mado-00-K2-G2-001_D03	265	protein	3.42624E-42	96.10%	F:structural constituent of ribosome; P:ribosome biogenesis; C:chloroplast stroma; C:cytosolic large ribosomal subunit; C:membrane; P:translation; F:rRNA binding
Mado-00-K2-G2-001_D05	346	rop family gtpase rop9	1.66989E-12	100.00%	C:nucleolus; C:phragmoplast; F:GTP binding; C:plasma membrane; F:protein binding; F:sphingomyelin phosphodiesterase activity; P:small GTPase mediated signal transduction; F:GTPase activity
Mado-00-K2-G2-001_D09	161	alpha-farnesene synthase	6.50203E-25	90.45%	P:metabolic process; F:lyase activity; F:magnesium ion binding
Mado-00-K2-G2-001_E03	242	phospho-2-dehydro-3-deoxyheptonate aldolase chloroplast	3.07671E-35	93.85%	F:3-deoxy-7-phosphoheptulonate synthase activity; P:aromatic amino acid family biosynthetic process; F:lyase activity; C:chloroplast; C:membrane
Mado-00-K2-G2-001_E04	344	swi snf complex subunit sw13	3.98993E-14	61.67%	F:identical protein binding; C:nucleus
Mado-00-K2-G2-001_E05	200	protein	7.17151E-24	94.75%	C:ribosome; F:structural constituent of ribosome; P:translation
Mado-00-K2-G2-001_E07	273	invertase pectin methylesterase inhibitor family protein	3.08752E-19	78.25%	F:pectinesterase activity; C:endomembrane system; P:shade avoidance; F:pectinesterase inhibitor activity
Mado-00-K2-G2-001_E08	387	like protein	2.13445E-12	62.10%	P:response to stress; C:nucleus; C:plastid
Mado-00-K2-G2-001_E09	115	f-box and wd40 domain	3.15064E-11	83.44%	P:auxin biosynthetic process; F:protein binding; P:protein import; P:intracellular protein transmembrane transport; F:ATP binding; P:protein targeting; F:transferase activity; C:membrane; F:zinc ion binding; C:plastid
Mado-00-K2-G2-001_E10	256	pip1b (named plasma membrane intrinsic protein 1b) water channel	2.6573E-34	90.85%	P:response to water deprivation; C:vacuole; P:water transport; C:plasma membrane; P:transmembrane transport; C:integral to membrane; F:water channel activity; C:chloroplast; C:mitochondrion; P:response to salt stress
Mado-00-K2-G2-001_F02	129	receptor serine-threonine protein	8.72391E-14	92.70%	P:auxin biosynthetic process; F:receptor activity; P:protein amino acid phosphorylation; F:non-membrane spanning protein tyrosine kinase activity; F:ATP binding; F:protein serine/threonine kinase activity
Mado-00-K2-G2-001_F04	362	high mobility group family	2.43027E-32	74.35%	C:nucleus; F:transcription factor activity
Mado-00-K2-G2-001_F05	257	endomembrane protein	8.31815E-12	96.85%	C:cytoplasmic membrane-bounded vesicle; C:integral to membrane; C:Golgi apparatus; C:mitochondrion; C:plasma membrane
Mado-00-K2-G2-001_F06	326	dna methyltransferase	2.01117E-34	71.65%	P:DNA methylation; C:chromatin; F:DNA (cytosine-5-)-methyltransferase activity; P:chromatin assembly or disassembly; F:DNA binding; F:chromatin binding; C:nucleus
Mado-00-K2-G2-001_F08	236	protein phosphatase	1.10183E-32	95.00%	C:protein serine/threonine phosphatase complex; F:metal ion binding; F:protein serine/threonine phosphatase activity; C:plasma membrane; P:protein amino acid dephosphorylation
Mado-00-K2-G2-001_G01	343	protein	9.45275E-32	76.75%	F:GTPase activity; F:GTP binding
Mado-00-K2-G2-001_G10	345	axial regulator	3.39441E-28	93.95%	P:regulation of transcription; F:transcription factor activity; C:nucleus
Mado-00-K2-G2-001_G11	159	s-adenosyl-l-homocysteine hydrolase	4.52374E-10	98.90%	F:adenosylhomocysteinase activity; P:one-carbon metabolic process; P:response to stress; F:binding
Mado-00-K2-G2-001_H02	238	glutathione reductase	9.93152E-34	94.30%	P:gamete generation; P:response to ionizing radiation; P:meiotic DNA double-strand break processing; P:cell redox homeostasis; P:oxidation reduction; P:glutathione metabolic process; F:glutathione-disulfide reductase activity; F:FAD binding; F:NADP or NADPH binding; C:cytoplasm
Mado-00-K2-G2-001_H04	338	protein	4.26436E-37	86.25%	C:chloroplast
Mado-00-K2-G2-001_H07	163	methionyl-trna synthetase	2.01949E-18	90.05%	C:cytosol; P:electron transport chain; F:electron carrier activity; F:methionine-tRNA ligase activity; F:ATP binding; F:tRNA binding; P:response to cadmium ion; P:methionyl-tRNA aminoacylation
Mado-00-K2-G2-001_H11	276	protein	2.18949E-41	99.00%	P:translation; P:ubiquitin-dependent protein catabolic process; P:response to UV-B; C:nucleolus; P:embryonic development ending in seed dormancy; F:structural constituent of ribosome; P:response to salicylic acid stimulus; P:protein ubiquitination; F:protein binding; C:vacuole; C:cytosolic large ribosomal subunit
Mado-00-K2-G2-001_H12	168	unnamed protein product [Vitis vinifera]	3.65697E-20	86.17%	F:molecular_function; P:biological_process

Mado-00-K2-G2-002_A03	155	wd-40 repeat protein	7.25121E-16	82.80%	F:receptor activity; C:heterotrimeric G-protein complex; F:myosin heavy chain kinase activity; P:response to cadmium ion; P:root development; P:shoot development; C:chloroplast; F:nucleotide binding
Mado-00-K2-G2-002_A06	229	c2 domain-containing protein	5.53129E-16	86.90%	C:chloroplast
Mado-00-K2-G2-002_B01	146	peptidyl-prolyl cis-trans isomerase	8.09291E-15	91.50%	P:defense response to bacterium; P:protein folding; C:chloroplast thylakoid lumen; F:polynucleotide adenylyltransferase activity; C:chloroplast stroma; F:peptidyl-prolyl cis-trans isomerase activity; C:chloroplast thylakoid membrane
Mado-00-K2-G2-002_B02	167	glucan endo- -beta-glucosidase	5.11921E-22	74.85%	F:glucan endo-1,3-beta-D-glucosidase activity; P:carbohydrate metabolic process; F:cation binding
Mado-00-K2-G2-002_B04	263	microtubule-associated protein eb1-like protein	5.69381E-29	80.80%	C:phragmoplast; P:positive gravitropism; F:microtubule binding; C:cortical microtubule, transverse to long axis; C:microtubule organizing center; C:preprophase band; P:thigmotropism; C:nucleus; C:spindle microtubule
Mado-00-K2-G2-002_B05	318	sphere organelles	1.97178E-13	70.36%	F:molecular_function; P:biological_process; C:cellular_component
Mado-00-K2-G2-002_B07	152	ribosomal protein s5	1.79166E-14	87.59%	C:ribosome; C:Golgi apparatus; F:structural constituent of ribosome; F:RNA binding; P:translation; C:plastid; C:mitochondrion
Mado-00-K2-G2-002_B10	159	60s ribosomal protein l19	5.33135E-19	97.15%	F:structural constituent of ribosome; C:plasma membrane; P:ribosome biogenesis; P:embryonic development ending in seed dormancy; C:cytosolic large ribosomal subunit; P:translation
Mado-00-K2-G2-002_C02	281	gasa4-like protein	4.15277E-8	74.84%	P:gibberellic acid mediated signaling pathway; F:protein binding
Mado-00-K2-G2-002_D03	350	chlorophyll a-b binding protein 4 precursor homolog	1.0323E-46	87.90%	C:plastoglobule; F:metal ion binding; P:photosynthesis, light harvesting; C:light-harvesting complex; C:photosystem II; P:protein-chromophore linkage; F:chlorophyll binding; C:integral to membrane; C:photosystem I; C:chloroplast thylakoid membrane
Mado-00-K2-G2-002_D10	225	wd-repeat protein	4.24954E-16	77.70%	C:CUL4 RING ubiquitin ligase complex
Mado-00-K2-G2-002_D11	285	mee9 (maternal effect embryo arrest 9)	1.62103E-15	83.67%	P:pollen development; P:embryonic development ending in seed dormancy
Mado-00-K2-G2-002_E05	351	tubulin beta-2 beta-3 chain	8.66484E-62	100.00%	P:microtubule-based movement; F:GTP binding; C:tubulin complex; P:response to cadmium ion; C:plasma membrane; F:protein binding; C:cell wall; P:protein polymerization; P:response to salt stress; C:mitochondrion; F:structural molecule activity; F:GTPase activity; C:microtubule
Mado-00-K2-G2-002_E08	302	60s ribosomal protein l30	1.63586E-36	95.45%	F:structural constituent of ribosome; C:cytosolic large ribosomal subunit; P:translation
Mado-00-K2-G2-002_E09	204	PREDICTED: hypothetical protein [Vitis vinifera]	2.29711E-14	86.21%	C:cytoplasmic membrane-bounded vesicle
Mado-00-K2-G2-002_E11	362	lipid binding	1.02105E-30	74.25%	C:cytoplasmic membrane-bounded vesicle
Mado-00-K2-G2-002_F01	152	elongation factor 1-	7.51003E-21	100.00%	C:cytoplasm; F:translation elongation factor activity; F:GTP binding; F:sulfate adenylyltransferase (ATP) activity; P:translational elongation; F:GTPase activity
Mado-00-K2-G2-002_F02	258	chalcone synthase	1.18589E-42	96.95%	F:acyltransferase activity; F:naringenin-chalcone synthase activity; P:flavonoid biosynthetic process
Mado-00-K2-G2-002_F03	305	60s ribosomal protein	6.63248E-30	97.95%	C:ribosome; F:structural constituent of ribosome; P:translation
Mado-00-K2-G2-002_F08	330	arginine methyltransferease	2.96198E-54	95.60%	C:cytoplasm; F:identical protein binding; P:protein amino acid methylation; F:histone-arginine N-methyltransferase activity; C:nucleus
Mado-00-K2-G2-002_G03	352	protein	7.17096E-24	78.75%	F:zinc ion binding; C:intracellular
Mado-00-K2-G2-002_G07	309	peptidoglycan-binding domain-containing protein	3.25985E-37	75.80%	C:cytoplasmic membrane-bounded vesicle; C:anchored to plasma membrane; P:cell wall macromolecule catabolic process
Mado-00-K2-G2-002_G08	263	qm-like protein	4.64704E-39	99.60%	C:ribosome; P:carbohydrate metabolic process; F:polygalacturonase activity; F:structural constituent of ribosome; P:translation
Mado-00-K2-G2-002_G10	173	histone h2a	2.28855E-14	94.90%	C:nucleosome; F:DNA binding; P:nucleosome assembly; C:nucleus
Mado-00-K2-G2-002_G12	329	predicted protein [Populus trichocarpa]	7.17592E-8	72.35%	C:plasma membrane
Mado-00-K2-G2-002_H01	279	exportin1 protein	1.58901E-34	89.65%	P:transmembrane transport; F:protein transporter activity; P:protein export from nucleus; P:embryo sac development; F:substrate-specific transmembrane transporter activity; F:receptor activity; C:nuclear pore; P:pollen tube growth; P:pollen development; F:protein binding; P:pollen germination; C:cytoplasm; P:protein import into nucleus, docking
Mado-00-K2-G2-002_H02	451	3-deoxy-d-arabino-heptulosonate 7-phosphate synthase	7.85169E-39	98.20%	F:3-deoxy-7-phosphoheptulonate synthase activity; P:aromatic amino acid family biosynthetic process; F:lyase activity; C:chloroplast; C:membrane
Mado-00-K2-G2-002_H04	254	dna methyltransferase	6.13323E-39	90.90%	P:chromatin modification; P:chromatin assembly or disassembly; P:regulation of transcription; C:plastid; F:DNA binding; C:chromatin; F:DNA (cytosine-5-)-methyltransferase activity; P:DNA methylation; F:chromatin binding; C:nucleus
Mado-00-K2-G2-003_A04	332	receptor-like kinase	2.43432E-40	96.95%	P:auxin biosynthetic process; F:receptor activity; P:protein amino acid phosphorylation; C:plasma membrane; F:protein binding; F:ATP binding; F:MAP kinase kinase kinase activity; C:integral to membrane
Mado-00-K2-G2-003_B01	410	protein	9.17036E-64	79.15%	P:carbohydrate metabolic process; C:integral to membrane; F:polygalacturonase activity; C:cell wall
Mado-00-K2-G2-003_B02	158	obtusifoliol 14-alpha demethylase	6.53779E-17	97.85%	F:sterol 14-demethylase activity; F:electron carrier activity; F:heme binding; P:embryonic development ending in seed dormancy; P:oxidation reduction; F:oxygen binding; F:methyltransferase activity; C:endoplasmic reticulum; P:sterol biosynthetic process
Mado-00-K2-G2-003_B03	244	protein	9.57563E-21	88.15%	C:plasma membrane; F:binding
Mado-00-K2-G2-003_C02	233	at4g31410 f8f16_230	2.6376E-26	70.95%	F:zinc ion binding; F:protein binding
Mado-00-K2-G2-003_C03	577	alpha tubulin 1	1.12146E-92	100.00%	P:microtubule-based movement; F:GTP binding; P:protein polymerization; F:structural molecule activity; F:GTPase activity; C:microtubule
Mado-00-K2-G2-003_D01	300	zinc finger	3.52162E-39	84.80%	C:endomembrane system; P:response to lithium ion; P:response to salicylic acid stimulus; F:carboxylesterase activity; P:hyperosmotic salinity response; P:lipid metabolic process
Mado-00-K2-G2-003_D02	272	60s acidic ribosomal protein p0	3.89247E-38	92.90%	C:cytosol; F:copper ion binding; C:nucleolus; P:response to salt stress; P:response to cold; F:structural constituent of ribosome; F:DNA-(apurinic or apyrimidinic site) lyase activity; P:translational elongation; P:ribosome biogenesis; P:response to zinc ion; C:chloroplast; P:response to cadmium ion; C:cytosolic ribosome; C:plasma membrane
Mado-00-K2-G2-003_D04	223	histone h2a	1.67674E-12	94.10%	P:defense response to bacterium; C:nucleosome; F:DNA binding; P:nucleosome assembly; F:protein binding; P:regulation of flower development; C:nucleus
Mado-00-K2-G2-003_D06	345	20s proteasome beta subunit pbc2	9.12058E-27	92.10%	C:proteasome core complex; C:cytoplasmic membrane-bounded vesicle; P:ubiquitin-dependent protein catabolic process; C:nucleus; F:threonine-type endopeptidase activity
Mado-00-K2-G2-003_E06	274	photosystem ii 10 kda chloroplast	5.61375E-29	85.80%	P:photosystem II oxygen evolving complex assembly; C:chloroplast thylakoid membrane; C:oxygen evolving complex
Mado-00-K2-G2-003_F01	295	ribulose- -bisphosphate carboxylase oxygenase small subunit	3.78137E-49	88.45%	P:reductive pentose-phosphate cycle; F:ribulose-bisphosphate carboxylase activity; F:monooxygenase activity; P:photorespiration; P:oxidation reduction; C:chloroplast
Mado-00-K2-G2-003_F02	432	eukaryotic initiation factor 4a	1.46207E-61	99.45%	P:auxin biosynthetic process; P:translational initiation; F:ATP-dependent helicase activity; P:response to cadmium ion; F:RNA binding; F:protein binding; C:cell wall; F:ATP binding; F:translation initiation factor activity; C:membrane; C:cytosol
Mado-00-K2-G2-003_F03	211	nucleic acid binding protein	1.58025E-15	89.70%	C:nucleus; F:nucleic acid binding; C:plasma membrane
Mado-00-K2-G2-003_F04	415	protein	3.33941E-66	82.25%	F:molecular_function; P:biological_process
Mado-00-K2-G2-003_F05	200	cell differentiation protein	1.82371E-27	98.20%	P:multicellular organismal development; C:plastid
Mado-00-K2-G2-003_G06	483	pyrophosphate-dependent phosphofructokinase alpha subunit	8.15108E-68	92.00%	C:pyrophosphate-dependent phosphofructokinase complex, alpha-subunit complex; P:response to sucrose stimulus; F:protein binding; F:6-phosphofructokinase activity; C:6-phosphofructokinase complex; F:diphosphate-fructose-6-phosphate 1-phosphotransferase activity; F:ATP binding; P:glycolysis; P:response to glucose stimulus; P:response to fructose stimulus; P:photosynthesis
Mado-00-K2-G2-003_H01	265	protein	6.90365E-43	93.30%	F:translation initiation factor activity; P:translation; P:transcription initiation; P:response to salt stress; P:photomorphogenesis; P:flower development; C:signalosome; C:eukaryotic translation initiation factor 3 complex; F:protein binding
Mado-00-K2-G2-003_H05	396	light-harvesting complex ii protein lhcb1	2.29099E-70	98.00%	F:metal ion binding; P:photosynthesis, light harvesting; C:photosystem II; P:protein-chromophore linkage; F:chlorophyll binding; C:integral to membrane; C:photosystem I; C:chloroplast thylakoid membrane
Mado-00-K2-G2-004B_A01	310	dead box atp-dependent rna	5.19237E-43	93.15%	P:auxin biosynthetic process; F:ATP-dependent helicase activity; F:nucleic acid binding; C:cell wall; F:ATP binding

Mado-00-K2-G2-004B_A04	353	protein	8.76591E-22	62.05%	C:vacuole; C:anchored to plasma membrane; F:copper ion binding; F:electron carrier activity; C:chloroplast; C:apoplast
Mado-00-K2-G2-004B_A10	369	protein phosphatase	2.38395E-59	92.70%	F:phosphoprotein phosphatase activity
Mado-00-K2-G2-004B_A12	151	acyl- -binding protein	1.91867E-8	85.20%	F:acyl-CoA binding
Mado-00-K2-G2-004B_B01	347	33kda precursor protein of oxygen-evolving complex	4.2073E-48	90.85%	C:chloroplast photosystem II; C:extrinsic to membrane; F:poly(U) RNA binding; F:oxygen evolving activity; F:calcium ion binding; P:regulation of protein amino acid dephosphorylation; F:protein binding; P:photoinhibition; C:chloroplast stroma; C:plastoglobule; P:photosystem II assembly; P:photosystem II stabilization; C:oxygen evolving complex; C:chloroplast thylakoid lumen
Mado-00-K2-G2-004B_B02	187	protein	6.57186E-25	81.75%	F:nucleic acid binding; F:ATP binding; F:helicase activity; C:chloroplast
Mado-00-K2-G2-004B_B04	131	endo- - -beta-d-	8.72391E-14	93.40%	F:carboxymethylenebutenolidase activity
Mado-00-K2-G2-004B_B05	251	protein	8.85941E-35	92.40%	C:respiratory chain complex I; P:photorespiration; C:mitochondrial membrane
Mado-00-K2-G2-004B_B07	162	protein	2.81796E-20	97.70%	C:membrane; F:catalytic activity
Mado-00-K2-G2-004B_B09	114	histone 3	1.84708E-11	100.00%	C:nucleosome; F:DNA binding; P:nucleosome assembly; C:nucleus
Mado-00-K2-G2-004B_C01	482	60s ribosomal protein l24	4.86072E-30	96.90%	P:gynoecium development; P:translation; F:structural constituent of ribosome; P:ribosome biogenesis; P:auxin mediated signaling pathway; C:cytosolic large ribosomal subunit; C:plasma membrane
Mado-00-K2-G2-004B_C02	224	hypothetical protein ARALYDRAFT_470811 [Arabidopsis lyrata subsp. lyrata]	3.37851E-13	100.00%	C:nucleosome; F:DNA binding; P:nucleosome assembly; C:nucleus
Mado-00-K2-G2-004B_C03	238	ribosomal protein s6 family protein	8.40753E-33	86.75%	C:ribosome; F:structural constituent of ribosome; P:ribosome biogenesis; P:translation; F:rRNA binding; C:chloroplast thylakoid membrane
Mado-00-K2-G2-004B_C04	119	udp-glucose pyrophosphorylase	8.266E-12	96.70%	C:cytoplasm; P:sucrose metabolic process; P:response to cadmium ion; C:plasma membrane; P:cellular response to phosphate starvation; F:UTP:glucose-1-phosphate uridylyltransferase activity; P:response to salt stress
Mado-00-K2-G2-004B_C05	232	60s ribosomal protein	5.665E-37	97.95%	C:ribosome; F:structural constituent of ribosome; P:translation; F:rRNA binding
Mado-00-K2-G2-004B_C06	177	nucleoside diphosphate kinase 2	1.57469E-15	93.10%	P:response to hydrogen peroxide; F:nucleoside diphosphate kinase activity; P:CTP biosynthetic process; P:red, far-red light phototransduction; P:auxin biosynthetic process; P:auxin mediated signaling pathway; P:response to UV; C:chloroplast stroma; F:identical protein binding; P:GTP biosynthetic process; P:UTP biosynthetic process; C:thylakoid; C:nucleus; F:ATP binding
Mado-00-K2-G2-004B_D01	218	gdp-mannose pyrophosphorylase	2.84742E-33	96.95%	P:defense response to bacterium; P:response to jasmonic acid stimulus; F:mannose-1-phosphate guanylyltransferase (GDP) activity; P:response to ammonium ion; P:response to ozone; P:cellulose biosynthetic process; F:mannose-1-phosphate guanylyltransferase activity; P:L-ascorbic acid biosynthetic process; P:response to heat; P:response to salt stress
Mado-00-K2-G2-004B_D04	177	elongation factor 1-alpha	6.37837E-25	100.00%	C:cytoplasm; F:translation elongation factor activity; F:GTP binding; P:translational elongation; F:GTPase activity
Mado-00-K2-G2-004B_D05	255	gtp-binding protein	3.35371E-37	97.20%	C:vacuole; F:GTP binding; C:plasma membrane; F:hydrolase activity; P:intracellular protein transport; C:trans-Golgi network; P:small GTPase mediated signal transduction
Mado-00-K2-G2-004B_D07	300	ubiquitin-activating enzyme	2.71524E-15	87.30%	P:auxin biosynthetic process; F:ubiquitin activating enzyme activity; C:plasma membrane; P:protein ubiquitination; P:ubiquitin-dependent protein catabolic process; F:ATP binding; F:ubiquitin-protein ligase activity; P:response to other organism
Mado-00-K2-G2-004B_D08	229	cystathionine gamma-synthase	1.54798E-34	91.00%	F:pyridoxal phosphate binding; F:cystathionine gamma-synthase activity; P:methionine biosynthetic process; F:lyase activity; C:chloroplast
Mado-00-K2-G2-004B_D11	305	histone 2	8.10765E-28	100.00%	P:defense response to bacterium; C:nucleosome; F:DNA binding; P:nucleosome assembly; F:protein binding; P:flower development; C:nucleus
Mado-00-K2-G2-004B_E02	186	iron ion binding acting on paired with incorporation or reduction of molecular 2-oxoglutarate as one and incorporation of one atom each of oxygen into both donors	2.95682E-17	74.44%	F:oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen; P:oxidation reduction; F:L-ascorbic acid binding; F:iron ion binding
Mado-00-K2-G2-004B_E03	211	maturase k	8.07836E-28	100.00%	P:mRNA processing; P:RNA splicing; C:chloroplast
Mado-00-K2-G2-004B_E07	201	cyclin-dependent kinase	1.10532E-16	82.25%	P:auxin biosynthetic process; P:regulation of G2/M transition of mitotic cell cycle; F:RNA polymerase II carboxy-terminal domain kinase activity; C:cyclin-dependent protein kinase holoenzyme complex; P:histone phosphorylation; F:ATP binding; P:hormone-mediated signaling pathway; F:protein binding; P:regulation of meristem structural organization; F:cyclin-dependent protein kinase activity
Mado-00-K2-G2-004B_E08	159	60s ribosomal protein l37	1.71504E-17	97.55%	F:metal ion binding; F:structural constituent of ribosome; P:ribosome biogenesis; C:cytosolic large ribosomal subunit; P:translation; F:rRNA binding
Mado-00-K2-G2-004B_E10	235	glycosyl hydrolase family protein	5.14197E-14	88.20%	C:endomembrane system; P:carbohydrate metabolic process; F:glucan endo-1,3-beta-D-glucosidase activity; F:cation binding
Mado-00-K2-G2-004B_F01	214	coclaurine n-methyltransferase	5.95719E-31	86.25%	F:cyclopropane-fatty-acyl-phospholipid synthase activity; P:lipid biosynthetic process; C:mitochondrion; C:plasma membrane; F:(S)-coclaurine-N-methyltransferase activity
Mado-00-K2-G2-004B_F02	253	band 7 family protein	1.05103E-22	77.75%	C:membrane; C:mitochondrion; F:zinc ion binding
Mado-00-K2-G2-004B_F05	276	elongation factor 1-	1.46522E-45	97.05%	C:cytoplasm; F:translation elongation factor activity; F:GTP binding; F:sulfate adenylyltransferase (ATP) activity; P:translational elongation; F:GTPase activity
Mado-00-K2-G2-004B_F06	229	histone h4	8.24628E-20	100.00%	C:nucleosome; F:DNA binding; P:nucleosome assembly; F:actin binding; P:cytoskeleton organization; C:nucleus; C:actin cytoskeleton
Mado-00-K2-G2-004B_F07	482	c2 domain-containing protein	2.38576E-72	96.00%	C:cell wall; C:endoplasmic reticulum
Mado-00-K2-G2-004B_F10	319	protein	1.82425E-51	87.75%	C:endomembrane system; P:steroid biosynthetic process; C:cell wall; F:methyltransferase activity
Mado-00-K2-G2-004B_F12	300	l-galactono- -lactone dehydrogenase	1.05787E-43	92.15%	F:L-gulono-1,4-lactone dehydrogenase activity; F:galactonolactone dehydrogenase activity; P:oxidation reduction; F:D-arabinono-1,4-lactone oxidase activity; P:L-ascorbic acid biosynthetic process; F:L-gulonolactone oxidase activity; C:integral to membrane; F:FAD binding; C:plastid; C:mitochondrial membrane
Mado-00-K2-G2-004B_G03	385	amidase family protein	1.2535E-20	82.90%	F:amidase activity; C:membrane; F:protein binding; F:carbon-nitrogen ligase activity, with glutamine as amido-N-donor; C:chloroplast
Mado-00-K2-G2-004B_G05	104	pointed first leaf	4.60475E-10	94.95%	C:nucleolus; C:vacuole; P:translational initiation; F:structural constituent of ribosome; C:plasma membrane; F:RNA binding; F:protein binding; C:cell wall; C:cytosolic small ribosomal subunit
Mado-00-K2-G2-004B_G08	226	geranylgeranyl pyrophosphate synthase-related protein	1.08065E-19	82.58%	P:diterpene phytoalexin metabolic process; P:isoprenoid biosynthetic process; F:farnesyltranstransferase activity; P:oxidation reduction; C:plastid; F:2-alkenal reductase activity
Mado-00-K2-G2-004B_H05	165	short-chain dehydrogenase reductase family protein	2.22571E-17	84.30%	F:3-oxoacyl-[acyl-carrier-protein] reductase activity; C:membrane; C:peroxisome; P:oxidation reduction; F:binding
Mado-00-K2-G2-004B_H09	462	anthocyanidin synthase	8.97931E-83	92.60%	P:flavonoid biosynthetic process; F:metal ion binding; F:L-ascorbic acid binding; P:oxidation reduction; F:oxidoreductase activity, acting on single donors with incorporation of molecular oxygen, incorporation of two atoms of oxygen; F:leucocyanidin oxygenase activity
Mado-00-K2-G2-004B_H10	346	uridylate kinase	1.0724E-43	93.05%	C:cytoplasm; F:adenylate kinase activity; P:nucleotide biosynthetic process; P:pyrimidine ribonucleoside monophosphate metabolic process; F:uridylate kinase activity; F:uridine kinase activity; F:ATP binding; F:cytidylate kinase activity; F:nucleoside triphosphate adenylate kinase activity

4 CAPÍTULO II

IDENTIFICAÇÃO, CLASSIFICAÇÃO E CARACTERIZAÇÃO TRANSCRICIONAL DAS DESIDRINAS DE MACIEIRA

Resumo expandido submetido ao XXII Congresso Brasileiro de Fruticultura

IDENTIFICAÇÃO, CLASSIFICAÇÃO E CARACTERIZAÇÃO TRANSCRICIONAL DAS DESIDRINAS DE MACIEIRA

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4.1 INTRODUÇÃO

Em um estudo de expressão gênica diferencial realizado anteriormente pelo grupo, a técnica de Hibridização Supressiva Subtrativa (SSH, do inglês, *Supression Subtractive Hybridization*) foi utilizada na identificação de genes potencialmente envolvidos na dormência de gemas em macieira (FALAVIGNA, 2010). Duas cultivares contrastantes quanto ao requerimento de frio (Gala e Castel Gala) foram utilizadas na construção de quatro bibliotecas subtrativas recíprocas na entrada e superação da dormência. Gemas da cultivar Gala, de maior requerimento de frio, apresentaram grande número de transcritos relacionados à resposta a estresses, tais como os codificadores de desidrinas.

As desidrinas (DHNs, do inglês, *dehydrins*) possuem sua expressão induzida por sinais ambientais relacionados à desidratação celular, tais como o frio, a salinidade e a seca. As DHNs atuam na proteção de membranas lipídicas celulares e conferem atividade crioprotetora em enzimas sensíveis ao frio (KOSOVÁ *et al.*, 2007). O acúmulo de determinadas DHNs em diversas espécies vegetais, inclusive em macieira, inicia durante o outono e alcança o seu maior nível no inverno, coincidindo com a máxima tolerância a temperaturas extremas de frio (FAUST *et al.*, 1997; FERNANDEZ *et al.*, 2012). As proteínas da família das DHNs são caracterizadas pela presença de um domínio altamente conservado, chamado de segmento K, o qual pode estar presente em uma ou mais cópias próximo à região carboxi-terminal. Outras sequências conservadas podem estar presentes, tais como o segmento Y e o segmento S. As DHNs são classificadas conforme o número de repetições desses segmentos (KOSOVÁ *et al.*, 2007).

A compreensão dos mecanismos que possibilitam que plantas superem condições ambientais adversas é de grande importância para a manutenção e a ampliação de culturas vegetais de interesse agronômico. Pelo presente estudo pretende-se identificar e classificar os genes codificadores de DHNs de macieira, bem como realizar a caracterização destes por meio de RT-qPCR.

4.2 MATERIAL E MÉTODOS

A sequência peptídica consenso para o segmento K das DHNs serviu de isca em buscas com a ferramenta *Basic Local Alignment Sequence Tool* (BLAST) nos genomas de macieira (*Malus* x *domestica* Borkh.) e pessegueiro (*Prunus persica* (L.) Batsch), disponíveis em www.rosaceae.org. O critério de seleção utilizado foi um *bit score* maior que 30 e 27, respectivamente. Três acessos de pessegueiro já haviam sido previamente caracterizados (BASSETT *et al*., 2009). Os acessos de *Arabidopsis* foram obtidos a partir do trabalho de BASSETT *et al*. (2009).

As sequências proteicas deduzidas dos 24 genes identificados foram submetidas à análise de domínios pelo programa *MEME Suite* v.4.8.1 (BAILEY *et al*. 1994). Parâmetros padrões foram utilizados, exceto o número máximo de motivos, definido para 10; o tamanho de cada motivo, definido entre oito e 25 aminoácidos; e o número mínimo de acessos, definido para sete.

As sequências peptídicas completas das DHNs foram alinhadas com o *software MUSCLE* (EDGAR, 2004). Uma árvore filogenética foi construída utilizando-se a inferência Bayesiana por meio do *software Mr. Bayes* 3.1.2 (HUELSENBECK *et al*. 2001). O tipo de substituição utilizado foi o misto, com 5.000.000 de gerações amostradas a cada 100, sendo as primeiras 250 árvores descartadas. As árvores restantes foram utilizadas para construir a árvore consenso.

Com base no genoma da macieira, 1.000 nucleotídeos anteriores ao códon de início de tradução de cada uma das *MdDHNs* foram utilizados para a caracterização da região promotora. A análise de elementos *cis* foi realizada por meio do banco de dados PLACE (Plant Cis-acting Regulatory DNA Elements; HIGO *et al*., 1999), buscando a identificação de elementos responsivos ao frio, desidratação e ácido abscísico (ABA).

Para a caracterização transcricional, oito amostragens de gemas fechadas de ‘Fuji *Standard*’ foram realizadas durante o ano de 2009 em um pomar da Estação Experimental da EPAGRI em Caçador, SC. RNA total foi purificado por precipitação diferencial em LiCl. A contaminação por DNA foi eliminada pelo tratamento com DNase I (Ambion) e os cDNAs foram sintetizados utilizando-se o *kit GeneAmp* (Applied Biosystems). Pares de *primers* específicos para *MdDHNs* foram projetados com os programas *Primer3 v.0.4.0* (ROZEN & SKALETSKY, 2000) e *OligoAnalyzer* (IDT). As RT-qPCRs foram realizadas no equipamento *StepOnePlus™ Real-Time PCR System* (Applied Biosystems) utilizando-se quantificação por fluorescência de *SYBR-Green* (1:100; Invitrogen). Cada amostra biológica (n=3) foi analisada em quadruplicata técnica. A amplificação consistiu em um *hot start* da enzima por 10 min a 95°C, seguido de 40 ciclos de (1)

desnaturação a 95°C por 15 s e (2) 60 s a 60°C para anelamento dos *primers* e extensão das fitas. Ao final, foi realizada a curva de dissociação entre as temperaturas de 60 e 95°C. A especificidade dos *primers* foi avaliada pela presença de pico único na curva de dissociação e banda única em gel de eletroforese. A expressão relativa foi calculada pelo método de PFAFFL (2001) em função dos genes referência *ARC5*, *MDH* e *WD40* (PERINI *et al*., dados não publicados).

4.3 RESULTADOS E DISCUSSÃO

A busca pelo segmento K nos genomas de macieira e pereira por BLAST permitiu a identificação das DHNs nestes organismos. Identificamos oito membros potencialmente codificadores de DHNs em macieira (genes *MdDHN2* a *9*), número condizente com outras espécies como *Arabidopsis* (com dez), pêssego (com seis), arroz e álamo (com nove; BASSETT *et al*., 2010).

A construção de um cladograma por meio de inferências Bayesianas permitiu a busca de homologias entre as sequências analisadas. Observou-se a formação de ortólogos e parálogos entre os acessos *MdDHN2* e *MdDHN5*; *MdDHN3* e *PpDHN2*; *MdDHN4* e *MdDHN7* com *PpDHN3*; *MdDHN6* e *PpDHN1*; e *MdDHN8* e *MdDHN9* (Figura 1).

A análise de domínios proteicos entre DHNs revelou a existência de diversos motivos conservados entre as sequências analisadas, os quais puderam ser relacionados com os segmentos já descritos. A classificação das sequências peptídicas deduzidas das MdDHNs foi realizada em função dos segmentos identificados (Figura 1). Entretanto, motivos ainda não descritos foram observados e demandam maiores estudos.

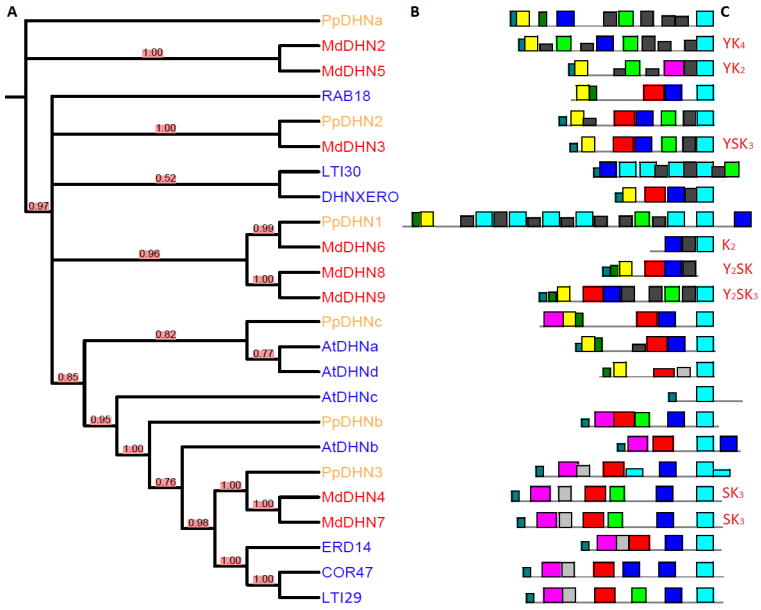

Figura 1 – Cladograma filogenético (A) e representação dos domínios de DHNs (B) de macieira (Md), pereira (Pp) e Arabidopsis (At, COR47, ERD14, LTI29, LTI30 e RAB18). O segmento K das DHNs está representado em azul claro, azul e verde claro em "B"; o segmento Y está representado em amarelo e verde escuro; o segmento S está representado em vermelho. Na coluna "C" está a classificação das MdDHNs quanto a presença dos segmentos conservados K, S e Y.

Elementos *cis* responsivos ao frio, desidratação e ABA regulam a expressão de genes *DHN*s em diversas plantas (BASSETT *et al.*, 2009). A busca de tais elementos foi realizada nos genes de *DHNs* de macieira. Os genes *MdDHN2*, *5* e *8* possuem sítios responsivos ao ABA (ABRE, do inglês, *ABscisic acid Responsive Element*). O gene *MdDHN3* possui elementos responsivos à desidratação (MYC e DRE, do inglês, *MYeloCytomatosis oncogene e Drought Responsive Element*, respectivamente), perfil consistente com seu ortólogo *PpDHN2*. Os genes *MdDHN4*, *6, 7 e 9* apresentaram elementos responsivos ao frio (CRT e ICEr2, do inglês *C-repeat* e *induction of CBF expression region 2*, respectivamente), mesmo perfil observado nos correspondentes ortólogos em pessegueiro (BASSETT *et al*., 2009).

O perfil transcricional obtido em gemas de macieira amostradas durante um ciclo anual de 'Fuji *Standard*' permitiu identificar um padrão similar de expressão entre os genes analisados. Observou-se um pico de concentração de transcritos no inverno para os oito *DHN*s de macieira, sendo possível relacionar a sua expressão com o processo de dormência e de aclimatação ao frio (Figura 2). A presença de diferentes elementos *cis* não resultou em perfis diferenciais de expressão, sugerindo a existência de mecanismos complementares de regulação gênica ainda não identificados. A menor e maior variação de expressão foi observada para os genes *MdDHN7* e *9*, respectivamente.

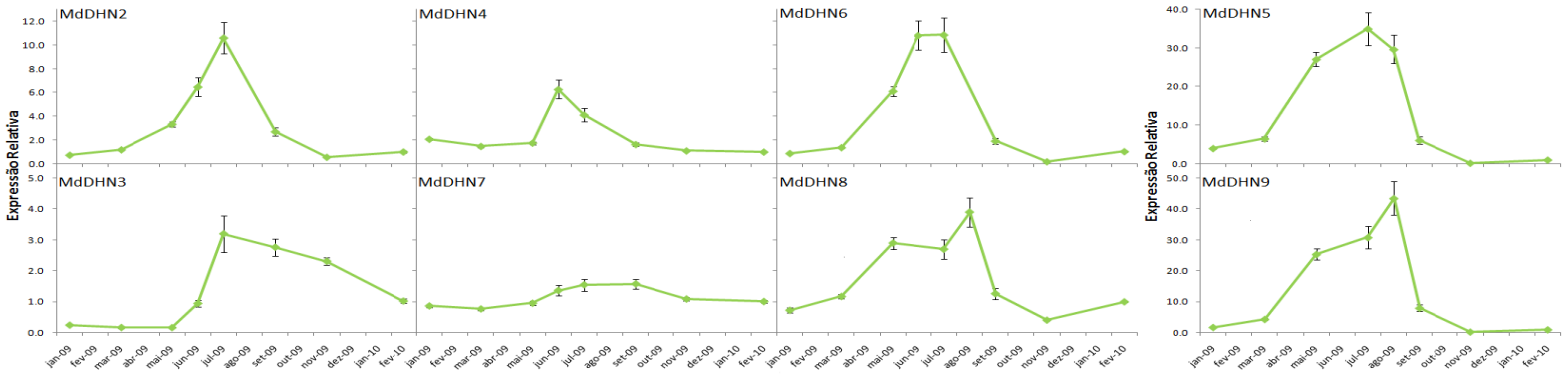

Figura 2 – Perfil transcricional por RT-qPCR das oito genes potencialmente codificadores de DHNs durante o ciclo anual de gemas de macieira. A expressão relativa em fevereiro de 2010 foi estabelecida como 1. As barras representam o erro padrão entre as replicatas biológicas. A quebra da dormência ocorreu em 15 de setembro de 2009.

4.4 CONCLUSÕES

Foram identificados oito potenciais genes de *DHN*s em macieira, os quais foram classificados conforme a presença dos segmentos conservados nas sequências peptídicas deduzidas. A análise da provável região promotora dos genes revelou elementos responsivos ao frio, à desidratação e ao ABA. Os oito genes exibiram um perfil sazonal de regulação da expressão em gemas de macieira. Esta é a primeira etapa de estudos voltados para uma melhor compreensão dos mecanismos moleculares de superação de condições ambientais adversas envolvendo DHNs.

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5 CONCLUSÕES

A utilização da técnica de hibridização supressiva subtrativa (SSH) mostrou-se eficiente na investigação da expressão gênica diferencial entre as cultivares Gala e Castel Gala de macieira, contrastantes para o requerimento de frio. Tal técnica, aliada aos adequados períodos de coleta de gemas escolhidos, evidenciou a presença de uma série de transcritos relacionados às mais variadas funções biológicas, as quais permitiram a caracterização de cada uma das quatro bibliotecas subtrativas obtidas. O modelo 'Gala' *vs* 'Castel Gala' permitiu, ainda, a identificação de um conjunto de genes diferencialmente expressos, os quais são candidatos a participarem dos processos de indução, manutenção e quebra da dormência de gemas em macieira.

A quantificação de transcritos por meio da técnica de RT-qPCR permitiu a validação dos genes candidatos previamente identificados por SSH. Dos 28 genes selecionados para tal, 17 destes apresentaram o mesmo perfil diferencial da biblioteca subtrativa de origem, tendo sido anotados como os genes codificadores de ADH, GAST, GoLS, três DHNs, duas histonas H2A variante H2A.Z, LTI65 (induzido por baixa temperatura 65, do inglês, *low temperature induced 65*) e os fatores de transcrição AP2, CAMTA1, DAM, GRAS, ICE1, NAC, RAP2.12 (relacionado ao APETALA2.12, do inglês, *Related to APETALA2.12*) e SCL (SCARECROW-like). Esta técnica também permitiu a realização da caracterização transcricional desse mesmo conjunto de genes ao longo de amostragens abrangendo o ciclo vegetativo e de dormência das cultivares Royal Gala, Castel Gala e Fuji *Standard*. Foram identificados genes com perfis sazonais de expressão, com acúmulo maior de transcrito nas cultivares de maior requerimento de frio, Royal Gala e Fuji *Standard*, em comparação com a de menor requerimento de frio, Castel Gala. Tal perfil é consistente com genes que possam estar atuando na indução e/ou manutenção do processo de dormência e aclimatação ao frio (JIMÉNEZ *et al.*, 2010). Dos 17 genes validados, transcritos codificando DAM, DHNs, GAST, LTI65, NAC, histonas variante H2A.Z e RAP2.12 apresentaram as maiores diferenças de expressão entre as cultivares durante o inverno e constituem-se como fortes candidatos a participantes do processo de progressão da dormência em macieira.

A família de genes *DHNs* de macieira teve seus membros identificados, classificados e caracterizados transcricionalmente. Oito potenciais genes codificadores de DHNs foram identificados em macieira. Por meio das suas prováveis sequências proteicas, estes foram

classificados conforme os segmentos peptídicos apresentados. Elementos *cis* responsivos ao frio, à desidratação e ao ABA foram identificados na provável região promotora dos genes *DHNs*, demonstrando que a expressão gênica dos mesmos pode estar envolvida na resposta a sinais ambientais sazonais. Os oito *DHN*s de macieira exibiram perfil sazonal de regulação da expressão, onde foi possível identificar um padrão similar entre os genes analisados, com um pico de acúmulo de transcritos durante o inverno. Esta é a primeira etapa de uma série de estudos voltados para uma melhor compreensão dos mecanismos moleculares de superação de condições ambientais adversas.

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7 CURRICULUM VITAE

DADOS PESSOAIS

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FORMAÇÃO

2011 – 2012

Mestrado em Biologia Celular e Molecular, PPGBCM, Universidade Federal do Rio Grande do Sul, UFRGS, Brasil. Perfil transcricional de genes relacionados à dormência em gemas de macieira. Orientadores: Dr. Giancarlo Pasquali e Dra. Márcia M.A.N.P. Margis.

2006 – 2010

Bacharelado em Engenharia de Bioprocessos e Biotecnologia. Universidade Estadual do Rio Grande do Sul, UERGS, Brasil. Expressão gênica diferencial entre duas cultivares de macieira com requerimento de frio hibernal contrastante. Orientadores: Dr. Fábio Luís Maciel, Dr. Diogo Denardi Porto e Dr. Luís Fernando Revers.

FORMAÇÃO COMPLEMENTAR

2010 – 2011

Estágio de Iniciação Científica no **Laboratório de Genética Molecular Vegetal**. EMBRAPA Uva e Vinho, Bento Gonçalves/RS.
Orientação: Dr. Luís Fernando Revers.
Pesquisa inserida no projeto: “Melhoramento genético de maçã: estratégias inovadoras no desenvolvimento de cultivares adaptadas às condições climáticas sul-brasileiras”.

2009 – 2009

Bolsista de ITI no **Laboratório de Biologia Molecular Vegetal**. EMBRAPA Uva e Vinho, Bento Gonçalves/RS.

Orientação: Dr. Luís Fernando Revers.

Atuando nos seguintes temas: Caracterização da dormência de gemas em macieira: obtenção de bibliotecas supressivas subtrativas para identificação de genes associados ao requerimento de frio hibernal; caracterização do nível de oxidação de glutationa durante a dormência hibernal em gemas de macieira; e busca por genes candidatos de macieira através de ferramentas da bioinformática.

2008 – 2009

Estágio de Iniciação Científica no **Laboratório de Enoquímica**. EMBRAPA Uva e Vinho, Bento Gonçalves/RS.

Orientação: Dr. Alberto Miele.

Atuando nos seguintes temas: Inovação tecnológica e rastreabilidade da produção de uvas e da qualidade dos sucos de uva e dos vinhos produzidos pelo sistema orgânico; avaliação do estado nutricional pelo método DRIS para culturas da macieira e videira no sul do Brasil; e efeito das mudanças climáticas na composição físico-química e nas características sensoriais do vinho fino.

PRÊMIOS E TÍTULOS

2011

Proficiência em leitura da Língua Inglesa, Universidade Federal do Rio Grande do Sul.

RESUMOS PUBLICADOS EM ANAIS DE CONGRESSOS

* MIOTTO, Y.E.; FALAVIGNA, V.S.; PORTO, D.D.; MARGIS-PINHEIRO, M.; PASQUALI, G.; REVERS, L.F. Identificação, classificação e caracterização transcricional

das desidrinas de macieira. *In:* XXII Congresso Brasileiro de Fruticultura, 2012, Bento Gonçalves. XXII CBF, 2012.

* FALAVIGNA, V.S.; PORTO, D.D.; ANZANELLO, R.; BUFFON, V.; SOUZA, D.A.; MARGIS-PINHEIRO, M.; PASQUALI, G.; OLIVEIRA, P.R.D.; CENTENO, D.C.; SANTOS, H.P.; REVERS, L.F. Transcriptional and metabolic profiling of two apple tree cultivars contrasting in chilling requeriment. *In:* 6th International Crop Science Congress, 2012, Bento Gonçalves. 6th ICSC, 2012.

* FALAVIGNA, V.S.; PORTO, D.D.; BUFFON, V.; MARGIS-PINHEIRO, M.; PASQUALI, G.; REVERS, L.F. Perfil transcricional em gemas dormentes de duas cultivares de macieira com requerimento de frio contrastante. *In:* 10º Encontro de Iniciação Científica e 6º Encontro de Pós-Graduandos da Embrapa Uva e Vinho, 2012, Bento Gonçalves. 10º Encontro de Iniciação Científica da Embrapa Uva e Vinho e 6º Encontro de Pós-Graduandos da Embrapa Uva e Vinho, 2012.

* PORTO, D.D.; FALAVIGNA, V.S.; BUFFON, V.; PASQUALI, G.; OLIVEIRA, P.R.D.; SANTOS, H.P.; REVERS, L.F. Differential gene expression of two apple cultivars with contrasting chilling requirement. *In:* III Simpósio Brasileiro de Genética Molecular de Plantas, 2011, Ilhéus/BA. III Simpósio Brasileiro de Genética Molecular de Plantas. São Paulo: Tech Art, 2011.

* FALAVIGNA, V.S.; PERINI, P.; REVERS, L.F. Identification of dormancy-associated mads-box genes in apple. *In:* III Simpósio Brasileiro de Genética Molecular de Plantas, 2011, Ilhéus. III Simpósio Brasileiro de Genética Molecular de Plantas. São Paulo: Tech Art, 2011.

* FALAVIGNA, V.S.; PORTO, D.D.; BUFFON, V.; OLIVEIRA, P.R.D.; SANTOS, H.P.; REVERS, L. F.; PASQUALI, G.; MARGIS-PINHEIRO, M. Caracterização do perfil transcricional associado ao estabelecimento e à superação da dormência de gemas entre cultivares de macieira contrastantes quanto ao requerimento de frio hibernal. *In:* XIII Reunião Anual do Programa de Pós-Graduação em Biologia Celular e Molecular, 2011, Porto Alegre. XIII Reunião Anual do Programa de Pós-Graduação em Biologia Celular e Molecular. Porto Alegre: UFRGS, 2011. v. 13. p. 134.

* MIOTTO, Y.E.; CUSIN, R.; FALAVIGNA, V.S.; PORTO, D.D.; REVERS, L.F. Identificação e análise de promotores de desidrinas no genoma da macieira. *In:* 9º Encontro de Iniciação Científica e 5º Encontro de Pós-Graduandos da Embrapa Uva e Vinho, 2011, Bento Gonçalves. 9º Encontro de Iniciação Científica e 5º Encontro de Pós-Graduandos da Embrapa Uva e Vinho. Bento Gonçalves: Embrapa Uva e Vinho, 2011.

* FALAVIGNA, V. S.; REVERS, L. F.; PEZZOTTI, M. Anotação funcional da família gênica Dof no genoma da videira. *In:* 9º Encontro de Iniciação Científica e 5º Encontro de Pós-Graduandos da Embrapa Uva e Vinho, 2011, Bento Gonçalves. 9º Encontro de Iniciação Científica e 5º Encontro de Pós-Graduandos da Embrapa Uva e Vinho. Bento Gonçalves: Embrapa Uva e Vinho, 2011.

* FALAVIGNA, V.S.; PORTO, D.D.; BUFFON, V.; PASQUALI, G.; OLIVEIRA, P.R.D ; SANTOS, H.P.; REVERS, L.F. Hibridização supressiva subtrativa aplicada à identificação de genes associados à dormência de gemas em macieira. *In:* 8º Encontro de Iniciação Científica da Embrapa Uva e Vinho e 4º Encontro de Pós-Graduandos da Embrapa Uva e Vinho, 2010, Bento Gonçalves. 8º Encontro de Iniciação Científica da Embrapa Uva e Vinho e 4º Encontro de Pós-Graduandos da Embrapa Uva e Vinho.. Bento Gonçalves: Embrapa Uva e Vinho, 2010.

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* PORTO, D.D.; FALAVIGNA, V.S.; BUFFON, V.; PASQUALI, G.; OLIVEIRA, P.R.D.; SANTOS, H.P.; REVERS, L.F. Differential gene expression of two apple cultivars with contrasting chilling requeriment. *In:* 5th International Rosaceae Genomics Conference (RGC5), 2010, Cape Town. 5th International Rosaceae Genomics Conference (RGC5). Cape Town, 2010.

* FALAVIGNA, V.S.; PORTO, D.D.; BUFFON, V.; BEKER, S.A.; REVERS, L.F. Caracterização da dormência de gemas em macieira: obtenção de bibliotecas supressivas subtrativas para identificação de genes associados ao requerimento de frio hibernal. *In:* 7º Encontro de Iniciação Científica da Embrapa Uva e Vinho e 3º Encontro de Pós-Graduandos da Embrapa Uva e Vinho, 2009, Bento Gonçalves. 7º Encontro de Iniciação Científica da Embrapa Uva e Vinho e 3º Encontro de Pós-Graduandos da Embrapa Uva e Vinho. Bento Gonçalves, 2009. p. 54-54.

PARTICIPAÇÃO EM CURSOS

* REVERS, L.F.; PORTO, D.D.; FALAVIGNA, V.S.; BUFFON, V.; MALABARBA, J. Genética Molecular Vegetal: mapeamento genético, identificação de QTLs, genes candidatos e suas aplicações tecnológicas. 2012. (Curso de curta duração ministrado/Extensão).