UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

CENTRO DE BIOTECNOLOGIA

PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA CELULAR E MOLECULAR

ESTRUTURA DO GENE *VvAGL11* E ANÁLISE DA EXPRESSÃO DURANTE A MORFOGÊNESE DA SEMENTE DE VIDEIRA

Dissertação de Mestrado

Jaiana Malabarba

Porto Alegre, outubro de 2014

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

CENTRO DE BIOTECNOLOGIA

PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA CELULAR E MOLECULAR

ESTRUTURA DO GENE *VvAGL11* E ANÁLISE DA EXPRESSÃO DURANTE A MORFOGÊNESE DA SEMENTE DE VIDEIRA

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 título de Mestre.

Jaiana Malabarba

Orientador: Prof. Dr. Giancarlo Pasquali

Porto Alegre, outubro de 2014

BANCA EXAMINADORA

_____

Prof.ª. Drª. Márcia M. A. N. Pinheiro-Margis

Programa de Pós-graduação em Biologia Celular e Molecular

Centro de Biotecnologia, Universidade Federal do Rio Grande do Sul

_____

Prof. Dr. Felipe dos Santos Maraschin

Departamento de Botânica, Instituto de Biociências

Universidade Federal do Rio Grande do Sul

_____

Prof. Dr. Marcelo Carnier Dornelas

Departamento de Biologia Vegetal,

Instituto de Biologia, Universidade Estadual de Campinas

"There are many hypotheses in science which are wrong.

That is perfectly all right: it's the aperture to finding out what's right.

Science is a self-correcting process."

Carl Sagan

"There's real poetry in the real world.

Science is the poetry of reality."

Richard Dawkins

AGRADECIMENTOS

ÍNDICE

LISTA DE ABREVIATURAS, SÍMBOLOS E UNIDADES

% - percentagem

3' - extremidade 3' -hidroxila de DNA ou RNA

5' - extremidade 5' -fosfato de DNA ou RNA

A – adenilato ou ácido adenílico

AG - gene *AGAMOUS*

AGL6 – gene *AGAMOUS-LIKE 6*

AGL11 – gene *AGAMOUS-LIKE 11*

AGL15 – gene *AGAMOUS-LIKE 15*

AGL17 – gene *AGAMOUS-LIKE 17*

AP1 – gene *APETALA 1*

AP2 – gene *APETALA 2*

AP3 – gene *APETALA 3*

AtAGL11 - gene *AGAMOUS-like* de *Arabidopsis thaliana*

AVAG2 - gene *AGAMOUS 2* de *Aspargus*

BPC1 - gene *BASIC PENTACYSTEINE 1*

BS - gene *B SISTER*

C – citidilato ou ácido citidílico

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

Col-0 - *Arabidopsis thaliana* acesso Columbia-0

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

DEF - gene *DEFICIENS*

DNase – desoxirribonuclease

EgMADS1 - gene *MADS-Box 1* de *Eustoma grandiflorum*

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

F1 - primeira geração filial (do inglês, *First Filial Generation*)

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

FBP7 - gene codificador da *FLORAL BINDING PROTEIN 7*

FBP11 - gene codificador da *FLORAL BINDING PROTEIN 11*

FLC - gene *FLOWERING LOCUS C*

FUL - gene *FRUITFULL*

G – guanidilato ou ácido guanidílico

INDEL- mutação de inserção ou deleção de nucleotídeos (do inglês *Insertion* e *Deletion*)

LMADS2 – gene *MADS-Box 2* de *Lilium*

Mcm1 - gene *minichromossome maintenance 1*

MAS - seleção assistida por marcadores (do inglês, *marker assisted selection*)

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

OIV - Organização Internacional da Uva e do Vinho (do francês, *Organization Internacional de la Vigne e du Vin*)

OsMADS13 - gene *MADS-Box 13* de *Oryza sativa*

pb – par (es) de bases

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

PhalAG2 - gene *AGAMOUS 2* de *Phalaenopsis*

PI - gene *PISTILATTA*

Place – do inglês, *Plant Cis-acting Regulatory DNA Elements*

QTL- *locus* de característica quantitativa (do inglês, *quantitatve trait locus*)

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

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

SdI - inibidor do desenvolvimento da semente (do inglês *Seed Development Inhibitor*)

SEP1 – gene *SEPALLATA 1*

SEP2 - gene *SEPALLATA 2*

SEP3 - gene *SEPALLATA 3*

SEP4 - gene *SEPALLATA 4*

SHP1 - gene *SHATTERPROOF 1*

SHP2 - gene *SHATTERPROOF 2*

SNP- polimorfismo de nucleotídeo único (do inglês *Single Nucleotide Polymorphism*)

SOC - gene *SUPPRESSOR OF OVEREXPRESSION OF CONSTANCE 1*

SRF - gene *SERUM RESPONSE FACTOR*

STK - gene *SEEDSTICK*

stk - mutante que não expressa o gene *STK/AtAGL11*

SVF - gene *SHORT VEGETATIVE PHASE*

T – timidilato ou ácido timidílico

UTR - região não traduzida (do inglês, *untranslated region*)

VvAG3 - gene *AGAMOUS 3* de *Vitis vinifera* (*VvAGL11*)

VvAGL11 - gene *AGAMOUS-LIKE 11* de *Vitis vinifera* (*VvAG3*)

WT - tipo-selvagem (do inglês, *wild-type*)

ZAG2 - gene *AGAMOUS 2* de *Zea mays*

ZMM1 - gene *MADS-BOX 1* de *Zea mays* (*ZMADS1*)

RESUMO

A videira é uma frutífera de origem antiga e atualmente é a mais cultivada do mundo, sendo de extrema importância econômica para diversos países. Certos genótipos de *Vitis vinifera* L., como a 'Sultanina', produzem bagas com apenas traços de sementes. Esta característica, denominada de apirenia, é classificada como estenoespermocarpia uma vez que a fecundação ocorre para a formação do fruto, porém é seguida de aborto do embrião devido à degeneração do endosperma. A apirenia é uma das características mais apreciadas para a uva de mesa e a compreensão dos mecanismos genéticos e moleculares controladores dessa característica é justificada pelo crescimento e pela exigência do mercado de uvas *in natura*. Estudos anteriores de nosso grupo permitiram identificar o gene *VvAGL11* como principal candidato atuante no controle do desenvolvimento de sementes em videira. Foi nosso objetivo, pelo presente trabalho, avaliar o gene *VvAGL11* nas cultivares pirênica Chardonnay e apirênica Sultanina, visando uma maior compreensão de seu papel durante a morfogênese da semente. Por meio de sequenciamento alelo-específico, foi possível caracterizar as variações alélicas do gene, o qual apresenta duas isoformas transcritas na cultivar pirênica Chardonnay. Em 'Sultanina', uma das isoformas identificadas possui mutações únicas. Pela avaliação do perfil transcricional de *VvAGL11* em diferentes estádios de desenvolvimento, da flor e do fruto, foi observado um grande acúmulo de transcritos em sementes de 2, 4 e 6 semanas em comparação com tecidos de flor e polpa. Na cultivar Sultanina, a expressão de *VvAGL11* foi extremamente baixa em todos os estádios e tecidos analisados. Análises morfo-anatômicas foram empregadas para comparar a semente e o traço de semente. Foram identificadas alterações anatômicas que sugerem a perda da identidade da camada de integumento médio do traço de semente de Sultanina, uma vez que esta camada não apresentou o padrão de diferenciação normal. O padrão de expressão espaço-temporal de *VvAGL11* foi determinado pela técnica de hibridização *in situ*. Foram utilizadas amostras das cultivares Chardonnay e Sultanina nos mesmos estádios de desenvolvimento analisados por RT-qPCR. Os níveis de transcritos de *VvAGL11* aumentaram significativamente em frutos de 'Chardonnay' com 2 e 4 semanas de desenvolvimento, especificamente na camada dupla do integumento médio da semente. Em 'Sultanina', não houve detecção de transcritos do gene em quaisquer estádios de desenvolvimento.

ABSTRACT

Grapevine have ancient origins and is currently the most widely cultivated fruit in the world, with high economic importance to many countries. Certain genotypes of *Vitis vinifera* L. like 'Sultanina' produce berries with only seed traces. This characteristic, termed apireny, is classified as stenospermocarpy since fertilization occurs aiming the formation of fruits but it is followed by the abortion of the embryo due to endosperm degeneration. Seedlessness is one of the most appreciated features for table grapes. Thereby the understanding of genetic and molecular mechanisms that control this feature is justified by the growth and the demand of *in natura* grapes' market. Our previous studies allowed us to identify a candidate gene, *VvAGL11*, possibly involved in the control of seed development in grapevine. Our objective with the present study was to evaluate *VvAGL11* in a pirenic cultivar, Chardonnay, and in an apirenic cultivar, Sultanina, in order to better understand its role during seed morphogenesis. By allele-specific sequencing, it was possible to characterize the allelic variations of *VvAGL11*, which exhibited two transcript isoforms in 'Chardonnay'. In 'Sultanina' one of the isoforms was identified with unique mutations. Evaluation of transcriptional profiles of *VvAGL11* during different developmental stages, from flowers to mature fruits, it showed a large accumulation of transcripts in seeds of 2, 4 and 6 weeks old compared to flower and pulp tissues in the Chardonnay cultivar. In 'Sultanina' the expression of *VvAGL11* was extremely low in all stages and tissues examined. Anato-morphological analyzes were performed in order to compare the seed and the seed trace. It was identified a loss of identity of the medium integument layer in the Sultanina seed trace since this layer neither elongated nor doubled in size as it should. The spatial-temporal expression pattern of *VvAGL11* was determined by an *in situ* hybridization technique. Samples of 'Chardonnay' and 'Sultanina' in the same stages of development analyzed by RT-qPCR were employed. *VvAGL11* transcript levels were significantly increased in 'Chardonnay' seeds with 2 and 4 weeks of development, specifically in the dual layer medium integument of the seed. In 'Sultanina', gene transcripts were undetectable during all stages of development.

1 INTRODUÇÃO

O desenvolvimento e a seleção de variedades de uva de mesa (*Vitis vinifera*) são considerados investimentos de longo prazo e de alto custo. A técnica de melhoramento clássico comumente utilizada envolve a geração de centenas de indivíduos F1 (do inglês, *First Filial Generation*) a cada ano, os quais necessitam de muito espaço para avaliação em campo e de longo período de cultivo para a obtenção de frutos (CABEZAS *et al*., 2006). Todo este processo gera perdas de produtividade pela demora excessiva, de até seis anos, para a avaliação da qualidade dos frutos. Visando a inovação deste sistema, a geração de plantas que apresentem caracteres de interesse por técnicas de engenharia genética é vantajosa para os grandes mercados produtores de frutíferas de desenvolvimento prolongado, como a videira, além da utilização de ferramentas biotecnológicas, como marcadores moleculares, que auxiliem na aceleração da geração e seleção de novas cultivares.

Muitos programas de melhoramento têm mantido o foco na geração de uvas de mesa que combinem ausência de sementes com outros caracteres de interesse agronômico no fruto tais como aumento do tamanho da baga, sabor moscatel e crocância (LOOMIS & WEINBERG, 1979; CAMARGO *et al*., 2005). A compreensão dos mecanismos genéticos e moleculares controladores da apirenia ou ausência de sementes em uvas de mesa possui grande relevância econômica, uma vez que o mercado consumidor desta fruta vem crescendo significativamente nas últimas décadas, além de ter aumentado sua preferência por uvas apirênicas (VITTI, 2009).

Diversos autores e grupos de pesquisa buscaram compreender as bases genéticas e moleculares da apirenia. A presença de um alelo dominante na região do *locus SdI* (Inibidor do desenvolvimento da semente, do inglês, *Seed development Inhibitor*), na extremidade do cromossomo 18 de *Vitis vinifera*, foi identificada como o principal QTL (*QTL*, do inglês, *Quantitative Trait Locus*) associado à apirenia, responsável por 50 a 70% da variância fenotípica total (BOUQUET E DANGLOT, 1996; LAHOGUE *et al*., 1998; DOLIGEZ *et al*., 2002; CABEZAS *et al*., 2006; MEJÍA *et al*., 2007; COSTANTINI *et al*., 2008). O gene *VvAGL11* (*AGAMOUS-LIKE 11*; acesso no genoma em http://genomes.cribi.unipd.it/grape/:Vv18s0041g01880) está localizado no mesmo *contig* do *Locus SdI* e foi proposto como um forte candidato responsável pela ausência de sementes

em videira (COSTANTINI *et al*., 2008; MEJÍA *et al*., 2011; REVERS *et al*., 2010). *VvAGL11* codifica um fator de transcrição do tipo MADS-box. Análises *in silico* permitiram sugerir uma possível ortologia entre *VvAGL11* e *SEEDSTICK* (*STK* ou *AtAGL11*) de *Arabidopsis thaliana* (acesso no genoma www.*Arabidopsis*.org: AT4G09960), o qual está envolvido no controle da identidade do óvulo (PINYOPICH *et al.,* 2003).

A identificação do gene *VvAGL11* localizado dentro de um *Locus* de Características Quantitativas significativamente associado à apirenia, aliado à sua ortologia com o gene *SEEDSTICK* de *Arabidopsis* justifica a caracterização deste gene no contexto da apirenia em videira. A compreensão de seu papel na morfogênese de sementes em uvas poderá ser útil no desenvolvimento de novas cultivares de uvas de mesa que atendam às exigentes demandas do mercado de consumo nacional e internacional.

1.1 MORFOGÊNESE DE SEMENTES E APIRENIA

1.1.1 Anatomia das Sementes em Desenvolvimento

As angiospermas apresentam uma estrutura especializada altamente complexa responsável pela propagação e manutenção de suas espécies, a semente. Esta é composta por três partes principais, cada qual com sua função: os integumentos, que protegem o endosperma e o embrião de situações adversas até que haja condições favoráveis para a germinação; o endosperma, que possui os nutrientes necessários para o desenvolvimento inicial do embrião; e o embrião, que se divide em duas partes, a radícula e o cotilédone, que germinarão para a formação de uma nova planta (Figura 1a; HAUGHN & CHAUDHURY, 2005).

As diversas camadas de integumentos que compõem a casca da semente (Figura 1b) possuem origem materna. Esta estrutura é formada, geralmente, por uma cutícula que se localiza acima de uma camada de epiderme e por três regiões tegumentares: integumento externo; uma camada dupla de integumento médio; e três camadas de integumento interno (PRATT, 1971; Figura 1b). Por outro lado, o endosperma e o embrião são gerados por dupla fecundação, sendo o primeiro triplóide, proveniente da fecundação da célula central

homodiplóide, e o segundo diploide, originado da fecundação da célula ovo haplóide (SITTE *et al.*, 2002).
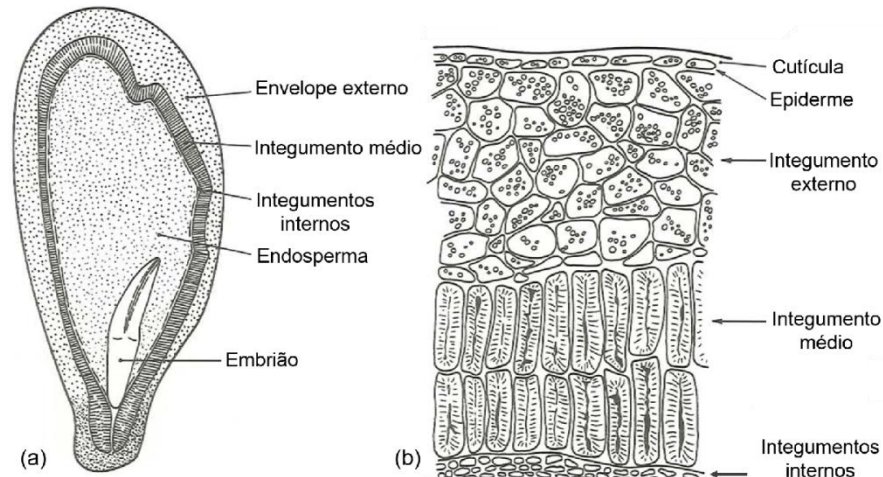
Figura 1. Ilustração da semente de *Vitis*. (a) A semente madura apresentada em corte longitudinal onde são visualizadas as camadas externas seguidas do integumento médio e de camadas de integumento interno. A porção central é composta pelo embrião circundado pelo endosperma. (b) Ilustração das camadas da casca da semente composta por uma camada de cutícula, uma camada de epiderme seguida pelo integumento externo e pelo integumento médio. Abaixo destes, iniciam-se as camadas de integumentos internos. Adaptado de PRATT (1971).

Em *A. thaliana*, o desenvolvimento da semente pode ser dividido em duas fases. A primeira é caracterizada pela proliferação e crescimento do endosperma, que aumenta o tamanho da semente em cerca de 100 vezes e traz grande contribuição para o seu tamanho final (VAROQUAUX *et al.*, 2000). Em contraste, o crescimento do embrião ocorre durante a segunda fase, quando há a expansão do endosperma (JÜRGENS & MAYER, 1994). O fluxo de nutrientes ocorre de forma centrípeta, do integumento externo para o interno, passando através de apoplastos para o endosperma e para o embrião em rotas que ainda não são completamente compreendidas (STADLER *et al.*, 2005). Ao fim da maturação da semente as camadas de integumentos morrem devido à sua lignificação, com exceção do endotélio (HAUGHN & CHAUDHURY, 2005). O crescimento da semente é, portanto, ditado pelo desenvolvimento conjunto do endosperma e dos integumentos (GARCIA *et al.,*

2005). Estudos já demonstraram que existe um forte efeito materno dos integumentos no controle e na determinação do tamanho da semente, por consequência de seu alongamento (BERGER *et al*. 2006). Deste modo, uma coordenação temporal e espacial de crescimento entre estes diferentes tecidos é crítica para o desenvolvimento correto da semente (UNGRU *et al*., 2010).

1.1.2 Apirenia

Os processos que regem o desenvolvimento de sementes estão bem caracterizados do ponto de vista fisiológico (EGLI, 1998). Diversas culturas que possuem apelo comercial voltado para a produtividade de grãos dependem deste conhecimento, bem como do seu entendimento do ponto de vista genético e molecular, para a obtenção de boas safras ano a ano. Entretanto, em culturas onde o produto final é o fruto, uma das características mais apreciadas pelos consumidores é a ausência de sementes.

Uma planta é considerada apirênica quando é capaz de produzir frutos sem sementes, com traços de sementes ou com sementes reduzidas em número e tamanho (VAROQUAUX *et al*., 2000). Duas formas de apirenia podem ser observadas dependendo do estádio em que a semente cessa o seu desenvolvimento, partenocarpia e estenoespermocarpia. Na partenocarpia, o ovário é capaz de se desenvolver em fruto sem que haja fecundação, apenas a partir do desenvolvimento dos tecidos maternos. Os frutos partenocárpicos são caracterizados pela ausência total de sementes. Por outro lado, na estenoespermocarpia, a fecundação ocorre normalmente e o desenvolvimento da semente se inicia. Porém, após o início de sua formação, ocorre aborto do embrião ainda imaturo devido à ausência ou má formação do endosperma, caracterizando a estenoespermocarpia pela produção de frutos com apenas traços de sementes (STOUT, 1936; BOUQUET & DANGLOT, 1996; VAROQUAUX *et al*., 2000; MEJÍA *et al*., 2011). Na maioria dos casos, o aborto do embrião ocorre oito semanas após a fecundação (EMERSHAD *et al*., 1989).

Frutíferas altamente comercializadas como videiras, laranjeiras, tomateiros e caquizeiros realizam, com mais frequência, a estenoespermocarpia. Este fator agrega valor aos frutos produzidos. Por consequência, os mecanismos moleculares que regem o cessar do

desenvolvimento da semente na estenoespermocarpia são de grande importância para que haja um entendimento maior deste fenótipo.

1.1.3   Mecanismos Moleculares da Morfogênese de Sementes

O desenvolvimento dos órgãos florais e o processo de transição floral são bem conhecidos do ponto de vista molecular (SMACZNIAK *et al*., 2012). Entre diversos componentes deste processo, os genes da família *MADS-box* possuem papel fundamental na regulação dos mecanismos que definem a identidade dos meristemas florais como sépalas, pétalas, estames, carpelos e óvulos, sendo que seus representantes são muito estudados na área de genética molecular vegetal com mais de 2.600 estudos publicados em revistas internacionais (SMACZNIAK *et al*., 2012).

1.1.3.1 Os genes *MADS-box*

Os fatores de transcrição da família MADS-box possuem papéis de extrema importância no desenvolvimento de plantas, pois são responsáveis pelo controle da identidade dos órgãos florais. A origem do nome MADS deve-se à alta similaridade desses fatores de transcrição com a proteína humana SRF (do inglês, *Serum Response Factor*; NORMAN *et al*., 1988), com o gene *Mcm1* (do inglês, *Minichromosome maintenance 1*) de *Saccharomyces cerevisiae* (PASSMORE *et al*., 1988), com o gene *AGAMOUS* (AG; YANOFSKY *et al*., 1990) de *A. thaliana* e com o gene *DEFICIENS* (DEF) de *Antirrhinum majus* (SOMMER *et al.,* 1990). Por este motivo, estes fatores de transcrição foram denominados de genes da família MADS (de **M**CM1, **A**G, **D**EF e **S**RF; SCHWARZ-SOMMER *et al*., 1990).

Os fatores de transcrição MADS-box contêm domínios de ligação ao DNA e de dimerização conservados em diversas espécies, chamados de domínio MADS (SCHWARZ-SOMMER *et al*., 1992; PELLEGRINI *et al*., 1995; HUANG *et al*., 2000). Os genes *MADS-box* são divididos em dois grupos tendo-se por base a estrutura dos seus domínios proteicos, denominadas de tipo I e de tipo II (SMACZNIAK *et al*., 2012). Os genes *MADS-box* de estruturas do tipo I constituem um grupo bem diversificado, contendo apenas ~180 pares de bases (pb) de sequências do domínio MADS em comum (DE BODT *et al*., 2003;

PARENICOVÁ *et al.*, 2003). Estudos recentes permitiram demonstrar que os genes pertencentes a este grupo possuem funções relacionadas com a gametogênese feminina e com o desenvolvimento de sementes (WUEST *et al.*, 2010).

Por outro lado, os genes *MADS-box* do tipo II foram mais bem estudados e caracterizados. Estes são genes homeóticos que atuam principalmente no desenvolvimento floral, na embriogênese e no desenvolvimento do fruto. Este grupo é caracterizado por sua estrutura denominada *MIKC*, na qual a porção N-terminal é composta pelo domínio MADS (M) de ligação ao DNA, seguido por dois domínios essenciais para a interação com proteínas, as porções I (*intervening*) e K (*keratin-like*). O domínio C-terminal é responsável pela formação e atividade de complexos proteicos e pela regulação transcricional (KAUFMANN *et al.*, 2005). Com base nas características estruturais deste domínio, os genes *MADS-box* do tipo *MIKC* foram ainda divididos em *MIKC^C* (forma canônica) e *MIKC**. Este último apresenta alterações no domínio K, possivelmente pela duplicação de éxons desta região, e forma uma rede de interação de proteínas responsável pela maturação do pólen (ADAMCZYK & FERNANDEZ, 2009).

Na planta-modelo *A. thaliana*, 107 genes *MADS-box* foram identificados nos últimos 20 anos, sendo que os genes do tipo *MIKC^C* compõem o modelo de desenvolvimento floral ABCDE (THEISSEN, 2001; THEISSEN & SAEDLER, 2001; PARENICOVA *et al.*, 2003; SMACZNIAK *et al.*, 2012). Os genes da classe A (*APETALA1 - AP1*) determinam a identidade de sépalas, os genes da classe A juntamente com genes da classe B (*APETALA3 - AP3 -* e *PISTILATTA - PI*) determinam a identidade das pétalas, ao passo que os genes da classe B somados aos genes da classe C (*AG*) são responsáveis pela formação de estames. Os genes da classe C possuem papel na identidade de carpelos (LOHMANN & WEIGEL, 2002), enquanto os genes da classe D (*SEEDSTICK-SHATTERPROOF1-SHATTERPROOF2*) atuam no desenvolvimento dos óvulos (Figura 2; COLOMBO *et al.*, 1995). Os genes *MADS-box* das classes A, B, C e D realizam interações com os genes da classe E (*SEPALATTA1-SEP2-SEP3-SEP4*), formando o modelo de quartetos florais (THEISSEN & SAEDLER, 2001 DORNELAS & DORNELAS, 2005). Interessantemente, as proteínas MADS-box formam complexos de alta ordem, normalmente em junções tetraméricas formadas por dois dímeros, como demonstrado na Figura 2.
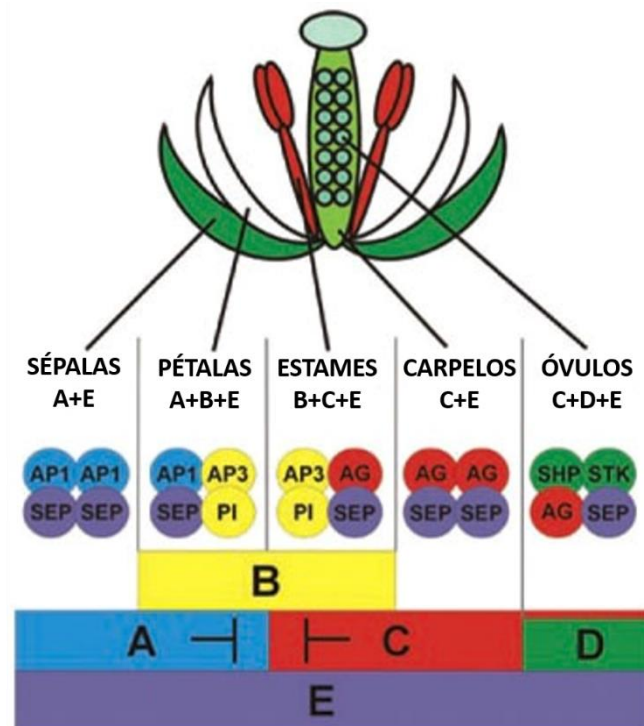
Figura 2. Modelo ABCDE de determinação da identidade dos órgãos florais por proteínas *MIKC^C* em *Arabidopsis thaliana*. Os genes *MIKC^C* da classe A (*AP1*) determinam a formação de sépalas; genes da classe B (*AP3* e *PI*), juntamente com genes da classe A, são necessários para a formação de pétalas. Os genes da classe B também são necessários para a formação de estames junto com os genes da classe C (*AG*), que por sua vez são necessários para a formação de carpelos. Os genes da classe D (*STK*, *SHP1* e *SHP2*) são responsáveis pelo desenvolvimento de óvulos. Todas as classes (ABCD) necessitam de, ao menos, um dos genes da classe E (*SEP1*, *SEP2*, *SEP3* e *SEP4*) para realizarem sua função. Adaptado de DORNELAS & DORNELAS (2005).

Os complexos quarternários das proteínas MADS-box normalmente ligam-se a duas sequências de DNA consenso denominadas CArG-box (CC(A/T)$_6$GG) separadas por até 300 pb (LIU *et al*., 2008). Essa interação entre complexos MADS-box e CArG-box é responsável pela geração de um *loop* de DNA entre os dois sítios CArG-box (THEISSEN, 2001; THEISSEN & SAEDLER, 2001; MENDES *et al*., 2013). A formação das diferentes interações proteicas determina a diferenciação e o crescimento dos órgãos florais criando, assim, inúmeras combinações de multimerização de proteínas que aumentam a diversificação de ligação dos complexos MADS-box ao DNA e, consequentemente, a

promoção da transcrição de genes por essas proteínas (MELZER & THEISSEN, 2009; SMACZNIAK *et al*., 2012).

1.1.3.2 Genes *MIKC^C* responsáveis pelo desenvolvimento de óvulo e de semente

Os genes *MIKC^C* das classes C e D formam a subfamília monofilética AG, pertencendo a clados irmãos uma vez que foram gerados por um evento de duplicação ocorrido no início da evolução das angiospermas (KRAMER *et al*. 2004). As proteínas codificadas por genes destas duas classes possuem dois motivos na região C-terminal conhecidos como AGI e II, os quais atuam na formação de complexos quaternários das proteínas MADS-box (YUN *et al*., 2004; SONG *et al*., 2006).

Os genes *MIKC^C* da classe D primeiramente caracterizados foram *FBP7* e *FBP11* (*FLORAL BINDING PROTEIN 7* e *11)* de *Petunia hybrida*, expressos especificamente nos óvulos. A geração de duplos mutantes destes genes levou à formação alterada destas estruturas (ANGENENT *et al*., 1995; COLOMBO *et al*., 1995; ANGENENT & COLOMBO 1996; COLOMBO *et al*., 1997). No estudo de COLOMBO *et al*. (1995), foi demonstrado que a expressão ectópica de *FBP7* ou de *FBP11* em *Petunia* induziu a formação de óvulos em pétalas e sépalas, mostrando o papel destes genes no desenvolvimento dos óvulos. Foram propostos ortólogos à *FBP7* e *FBP11* em outras espécies como *AGL11* (*STK*) em *A. thaliana* (ROUNSLEY *et al*., 1995; PINYOPICH *et al*., 2003), *ZAG2* e *ZMM1* em *Zea* (SCHMIDT *et al*., 1993; THEISSEN *et al*., 1995), *OsMADS13* em *Oryza* (LOPEZ-DEE *et al*., 1999), *PhalAG2* em *Phalaenopsis* (SONG *et al*., 2006), *AVAG2* em *Asparagus* (YUN *et al*., 2004), *LMADS2* em *Lilium* e *EgMADS1* em *Lisianthus* (TZENG *et al*. 2002). Todos estes genes tendem a especificar a identidade dos tecidos da quarta camada da flor.

Em *A. thaliana*, o gene *AtAGL11* é responsável pela identidade do óvulo juntamente com os genes *SHATTERPROOF 1* e *SHATTERPROOF 2* (*SHP1, SHP2*), e seu mutante defectivo apresenta fenótipo de sementes diminutas (PINYOPICH *et al*., 2003; BRAMBILLA *et al*., 2007). O gene *AtAGL11* é responsável pela expansão e divisão celular do funículo, sendo que a ausência deste gene faz com que esta estrutura se torne mais larga e espessa (ROUNSLEY *et al*.,1995; PINYOPICH *et al*., 2003). Este gene é uniformemente

expresso nos integumentos dos óvulos e ao longo do funículo nas fases iniciais de desenvolvimento da semente. Na fase de maturação, *AtAGL11* é fortemente expresso no funículo e fracamente nos integumentos (ROUNSLEY *et al.*, 1995; PINYOPICH *et al.*, 2003; MATIAS-HERNANDEZ *et al.*, 2010). Seu mutante (*stk*), além de demonstrar diminuição no tamanho das sementes e aumento da espessura do funículo (Figura 3d-e), possui síliquas menores com apenas metade da quantidade normal de sementes (Figura 3a). O mutante *stk* também apresenta o fenótipo de não abscisão das sementes (Figura 3f), provavelmente pela estrutura diferenciada do funículo que as mantém fortemente ligadas à rafe (PINYOPICH *et al.*, 2003).
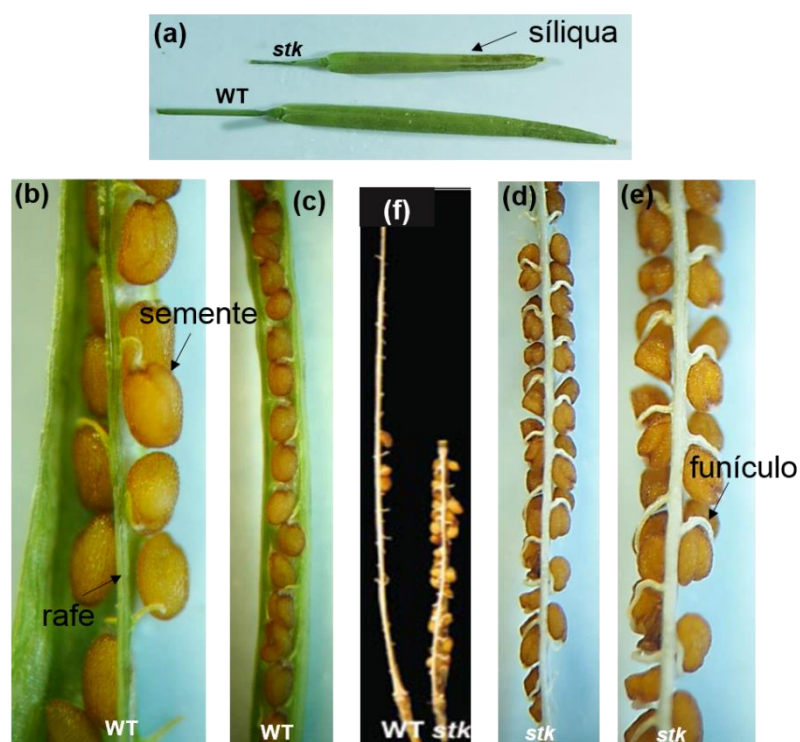
Figura 3. Comparação entre *Arabidopsis thaliana* Col-0 selvagem (WT, do inglês, *wild-type*) e *A. thaliana* Col-0 mutante para o gene *AtAGL11* (*stk*). (a) Diferença entre o tamanho de síliqua; (b-c) formato e tamanho da semente de WT e espessura e comprimento do funículo de WT; (d-e) formato e tamanho da semente de *stk* e espessura e comprimento do funículo de *stk*; (f) diferença entre WT e *stk* em relação à abscisão das sementes da rafe. Imagem f realizada por PINYOPICH *et al.* (2003).

A regulação da expressão tecido-específica do gene *AG* é dependente de elementos *cis* presentes no seu segundo íntron, conhecido por sua grande extensão (3 quilobases ou kb, do inglês, *kilobase*) (DEYHOLOS 2000; HONG *et al* 2003). Da mesma maneira, o gene *AtAG11* (*STK*) é regulado por elementos *cis* localizados em sua região promotora e em seu grande primeiro íntron (1,3 kb). Esses elementos regulatórios são ricos em G-A, no qual a proteína BASIC PENTACYSTEINE 1 (BPC1) se liga e gera uma mudança conformacional do DNA que auxilia na formação do precomplexo de ativação da transcrição deste gene (KOOIKER *et al*., 2005).

A compreensão da regulação da expressão de genes responsáveis pelo desenvolvimento de semente em plantas modelos como *A. thaliana* avançou significativamente nos últimos anos (MATIAS-HERNANDEZ et al., 2010). Do mesmo modo, estudos abordando este tema também vêm sendo conduzidos em outras espécies tais como videira, tomateiro, laranjeira, entre outros (VAROQUAUX *et al*., 2000). Uma das plantas que têm sido extensivamente estudadas com o intuito de buscar uma maior compreensão do processo de apirenia é a videira. Nesta planta, a subfamília AG foi previamente caracterizada, sendo composta por três membros, dois deles mais similar a *AG* (*VvAG1 e VvAG2*) e um terceiro, *VvAGL11* ou *VvAG3,* mais similar de *AGL11/STK* (BOSS *et al*., 2001 e 2002). Porém, nenhum gene foi identificado como ortólogo de *SHP1* e *SHP2* (DÍAZ RIQUELME *et al*., 2009). A confirmação da ortologia entre *AtAGL11* e *VvAGL11* auxiliaria no processo de compressão da ausência de semente em *V. vinifera.*

1.2 A CULTURA DA VIDEIRA

A videira (*Vitis* spp. L.) é uma planta de origem antiga, proveniente de áreas caucasianas e que se propagou inicialmente na área mediterrânea. Sua grande expansão mundial ocorreu graças a sua habilidade de se adaptar a diversos solos e condições climáticas (LACIRIGNOLA & DIGIARO, 1999). Atualmente, a videira é a frutífera mais cultivada mundialmente, cobrindo 7,8 milhões de hectares em 2011 e sendo a 5ª fruta mais produzida no mundo com 67,5 milhões de toneladas de frutos em 2012 (OIV, 2011; FAO, 2012). A videira possui grande apelo comercial uma vez que as suas bagas são utilizadas para as mais diversas finalidades tais como produção de vinhos (68%), consumo de uvas de mesa *in natura* (30%), produção de uvas passas (2%) e produtos de menor impacto como sucos,

geleias, etanol, vinagre, óleo de sementes, ácido tartárico e fertilizantes (FASOLI *et al*., 2012).

A viticultura é uma atividade tradicional em países de clima temperado e, recentemente, tem ganhado grande significância em diversas regiões de clima subtropical e tropical. Esta característica abre perspectivas para uma grande expansão na viticultura tropical, principalmente para a produção de uvas de mesa (AMARAL *et al*., 2012). A produção mundial de uva de mesa atingiu 2,1 milhões de toneladas em 2010, sendo que o Brasil ocupa o 6° lugar na produção mundial de uva de mesa (FAO, 2010).

A apirenia é uma das características mais apreciadas em uvas de mesa, em concomitância com frutos de tamanho elevado. Estas duas qualidades constituem principais objetivos de programas de melhoramento de uvas de mesa (VAROQUAUX *et al*., 2000; CABEZAS *et al*., 2006). Nos Estados Unidos da América, as uvas apirênicas já dominam o mercado enquanto que, na Europa, é crescente a demanda por uvas sem sementes. Dados de 2012 da Organização Internacional da Uva e do Vinho (OIV, do francês, *Organisation Internationale de la Vigne et du Vin* e da Organização das Nações Unidas para Agricultura e Alimentação (FAO, do inglês, *Food and Agriculture Organization of the United Nations*), permitiram comprovar que nas últimas duas décadas a comercialização mundial de uva *in natura* aumentou cerca de 26% ao ano, enquanto que a produção de uvas para esta finalidade cresceu a taxas anuais próximas de 13%. Essa expansão comercial gerou oportunidades de crescimento aos países produtores e a competitividade entre os mercados exportadores das melhores uvas apirênicas (LAZZAROTTO & FIORAVANÇO, 2012).

A produção de uvas brasileiras *in natura* está baseada, predominantemente, na cultivar Niágara Rosada, no caso de uvas de mesa comuns. Em contrapartida, as uvas finas de mesa são, essencialmente, as cultivares Itália, Rubi, Benitaka, Brasil e Red Globe, que são uvas pirênicas, e as cultivares Centennial Seedless, Superior Seedless, Sultanina, Catalunha e Crimson Seedless, que são uvas apirênicas (PROTAS, 2005). Dentre estas, a cultivar Sultanina destaca-se devido à sua boa adaptação ao cenário europeu e estado-unidense de cultivo, onde foi amplamente utilizada em cruzamentos visando a geração de novas cultivares apirênicas e, por isso, vêm sendo estudada como modelo de apirenia em videira.

1.2.1 A Cultivar Sultanina

Atualmente, diversas variedades de uvas apirênicas são produzidas no mundo, cada qual sendo escolhida por sua adaptabilidade às condições locais. As principais cultivares estenoespermocárpicas são, em sua maioria, descendentes de 'Sultanina', também chamada de 'Thompson Seedless'. Esta é uma das cultivares mais importantes comercialmente e é, até hoje, utilizada em cruzamentos como doadora de seu fenótipo apirênico (ADAM-BLONDON *et al*., 2001; GENOVA *et al*., 2014). Na Figura 4, é possível visualizar o contraste entre uma cultivar pirênica, neste caso 'Chardonnay', e 'Sultanina', que é estenoespermocárpica e, portanto, origina frutos com traços de sementes pouco desenvolvidos e macios, imperceptíveis ao consumidor, sendo fortemente apreciada no mercado de uvas de mesa (STOUT, 1936). A mutante somática natural Sultanina possui coloração mais clara e estava entre um grupo de variedades inicialmente cultivadas em Nova York e, em 1872, em Marysville, Califórnia, por Willian Thompson do qual descende seu nome mais comum. Originalmente, a cultivar Sultanina é proveniente da Pérsia da Ásia Menor, atualmente áreas do Irã e da Turquia, onde era conhecida como 'Oval Kechmish' (CHRISTENSEN, 2000).

Para a determinação do fenótipo de apirenia de uma determinada cultivar de videira é utilizada uma classificação determinada pela OIV sobre a norma descritora 243 (Figura 5). De acordo com esta norma utiliza-se dados de matéria seca de 100 sementes aleatoriamente amostradas, classificadas pela seguinte ordem: classe 1 - muito baixa ($\leq$10 mg/semente); classe 3 - baixa (21-29 mg/semente); classe 5 - média (36-44 mg/semente); classe 7 - alta (51-59 mg/semente) e classe 9 - muito alta (>65 mg/semente) como mostrado na Figura 6. As variedades que se encaixam nas classes 1, 3 e 5 são consideradas apirênicas, enquanto que as cultivares com notas 7 e 9 são consideradas pirênicas.
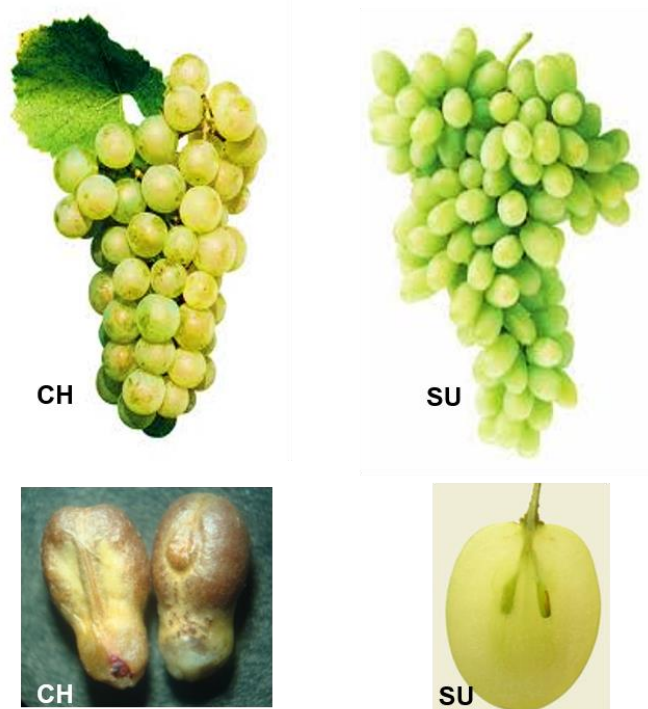
Figura 4. Comparação entre a cultivar pirênica Chardonnay (CH) e a cultivar apirênica Sultanina (SU), ambas em estádio de maturação dos frutos. 'Chardonnay' apresenta sementes de tamanho adequado e lignificadas enquanto que 'Sultanina' (baga) contém apenas o traço de uma semente. Imagem de 'Chardonnay' proveniente de Ternuta dei Mille (2011), e de 'Sultanina' proveniente de Agrofresh (2013). Semente de 'Chardonnay' com aumento de 40X.
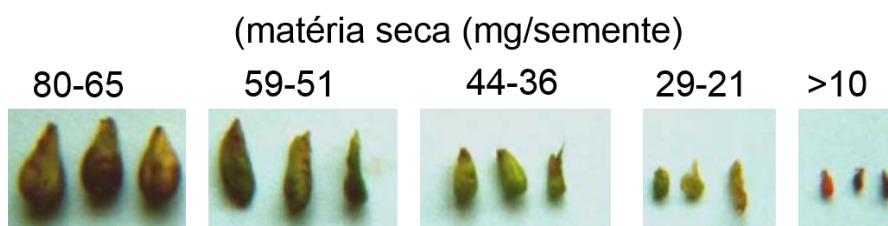
Figura 5. Classificação da pirenia e apirenia em videira segundo o descritor OIV 243. Distribuição das classes fenotípicas para a determinação de apirenia. Peso de matéria seca entre 44 - >10 mg/semente, abrangendo as classes 1, 3 e 5, classifica a cultivar como apirênica. Peso de matéria entre 51 - < 65, classes 7 e 9, determina que a cultivar é pirênica. Imagem proveniente de Acervo EMBRAPA Uva e Vinho. Adaptado de BOUQUET & DANGLOT (1996).

1.2.2 Controle Genético e Molecular da Apirenia em Videira

V. vinifera possui seu genoma completamente sequenciado em sua forma quase homozigota e, também, em sua forma altamente heterozigota, ambas obtidas a partir da cultivar Pinot Noir (PN40024; JAILLON *et al*., 2007; VELASCO *et al*., 2007), caracterizando o genoma-referência de videira (http:// http://genomes.cribi.unipd.it/grape/). Este conjunto de dados genômicos auxilia em estudos de desenvolvimento de cultivares por métodos não tradicionais, concomitantemente com o entendimento da função de seus mais de 23 mil genes.

A identificação e a compreensão dos mecanismos genéticos e moleculares que regem o desenvolvimento de espécies com ausência de sementes são essenciais para a obtenção de culturas comerciais de alta qualidade e competitividade no mercado (CAMARGO *et al*., 1999). Como alternativa biotecnológica, muitos pesquisadores focaram seus esforços na busca de marcadores moleculares que pudessem ser utilizados para a detecção do fenótipo de apirenia prematuramente, utilizando-os em estratégias de seleção assistida por marcadores (MAS, do inglês, *Marker Assisted Selection*) em populações segregantes (STRIEM *et al*., 1996; LAHOGUE *et al*., 1998; ADAM-BLONDON *et al*., 2001; MEJÍA *et al*., 2007; MEJÍA *et al.,* 2011).

O método de obtenção de mapas genéticos baseados na estratégia do duplo pseudocruzamento-teste, desenvolvido por GRATTAPAGLIA & SEDEROFF (1994), permitiu identificar QTLs com grandes efeitos fenotípicos. Esta estratégia é muito utilizada para espécies altamente heterozigotas e de demorado desenvolvimento como *V. vinifera*, sendo que os indivíduos avaliados são a progênie do cruzamento, plantas F1. Deste modo, a produção de mapas genéticos de videira aumentou consideravelmente (LODHI *et al*., 1995; DALBO *et al*., 2000; DOLIGEZ *et al*., 2002; RIAZ *et al*., 2003; ADAM-BLONDON *et al*., 2004; FISCHER *et al*., 2004; CABEZAS *et al*., 2006).

Diversos graus de tamanho de sementes podem ser observados em cultivares de videiras estenospermocárpicas e em suas progênies, o que demonstra que a estenoespermocarpia é um caractere quantitativo. BOUQUET & DANGLOT (1996) geraram um modelo de controle desta característica. Neste modelo, alelos recessivos de três *loci* independentes são regulados por um alelo dominante em um único *locus*, o qual inibiria

o desenvolvimento da semente. Posteriormente, este *locus* foi denominado *SdI* (Inibidor do desenvolvimento da semente, do inglês, *Seed development Inhibitor*) (LAHOGUE *et al*., 1998).

Em estudos genéticos com populações geradas a partir de variedades de videira pirênicas (doadora materna) e apirênicas (doador paterno), foram detectados QTLs para tamanho de baga, peso e tamanho de sementes em *V. vinifera*. Os QTLs estão localizados no grupo de ligação 18, na porção distal do cromossomo, e explica de 50 a 70% da variância fenotípica para esses caracteres (DOLIGEZ *et al*., 2002; FANIZZA *et al*., 2005; CABEZAS *et al*., 2006; MEJÍA *et al*., 2007; CONSTANTINI *et al*., 2008; REVERS *et al*., 2010). Este QTL corresponde ao *locus SdI* proposto anteriormente no modelo de BOUQUET & DANGLOT (1996) (MEJÍA *et al*., 2011).

A partir deste *QTL,* o gene *AGAMOUS-LIKE 11* (*VvAGL11*) foi mapeado *in silico* no mesmo *contig* que o *locus SdI* e também colocalizou com o marcador molecular SSR (repetição de sequência simples, do inglês, *Simple Sequence Repeat*) VMC7F2, que se encontra 463 pb a montante do códon de início de tradução de *VvAGL11*. Este marcador é altamente polimórfico, identificando diferenças entre os alelos do gene *VvAGL11* por amplificar uma região que apresenta tamanhos desiguais. Assim, é possível detectar cada um dos dois alelos de 'Sultanina', no caso um dos alelos é idêntico ao do genoma referência ('Pinot Noir') e é chamado de alelo selvagem, enquanto que o outro alelo, chamado de alelo mutado, possui diferenças em sua região promotora. VMC7F2 é utilizado com 95% de eficácia para detecção de apirenia em videira (CONSTANTINI *et al*., 2008). Pelo emprego deste marcador na técnica de MAS é possível identificar o genótipo de cada indivíduo, podendo este ser homozigoto para o alelo normal (pirênico), heterozigoto (apirênico) ou homozigoto para o alelo mutado (apirênico).

VvAGL11 (acesso no genoma em http://genomes.cribi.unipd.it/grape/: Vv18s0041g01880), também chamado por BOSS *et al*. (2002) de *VvMADS5* e por DÍAZ-RIQUELME *et al*. (2009) de *VvAG3*, é um possível ortólogo do gene *AtAGL11/STK* de *A. thaliana* que, por sua vez, atua no controle da identidade do óvulo (FAVARO *et al*., 2003; PINYOPICH *et al*., 2003). Em um estudo contemplando a família MADS-box em videira, DÍAZ-RIQUELME *et al*. (2009) demonstrou que *VvAGL11* é induzido em tecidos florais e em frutos, enquanto que é reprimido em raiz, ramo, folha, gavinha e gema. Ainda nesse estudo, foi demonstrada uma possível ortologia entre *VvAGL11* (*VvAG3* na Figura 6),

AtAGL11 e *PMADS21* de álamo. Estes genes agruparam-se na subfamília AG (Figura 6), na qual os genes *MADS-box MIKC* de videira, álamo e *A. thaliana* foram agrupados em uma árvore filogenética que se mostrou dividida em 13 subfamílias (SEPALLATA, AGAMOUS-LIKE 6, APETALA1-FRUITFULL, FLOWERING LOCUS C, AGAMOUS, AGAMOUS-LIKE 12, TM 8, AGAMOUS-LIKE 15, AGAMOUS-LIKE 17, SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1, SHORT VEGETATIVE PHASE, APETALA-PISTILLATA, B SISTER) dentro de 10 clados maiores.
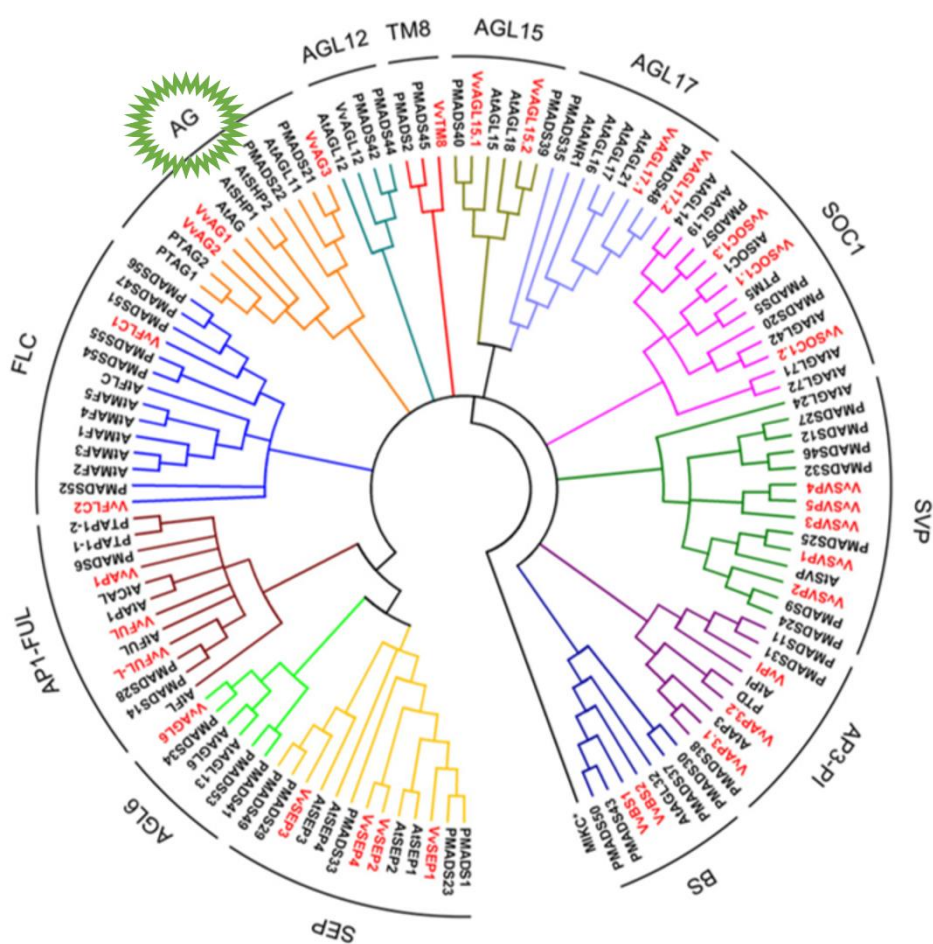
Figura 6. Árvore filogenética da família de genes *MIKC* de *Vitis* (em vermelho), *Arabidopsis* e *Populus*. Árvore gerada pelo alinhamento das sequências completas de aminoácidos das proteínas de cada gene. O método junção de vizinhos (NJ, do inglês, *neighbor-joining*) foi utilizado para o alinhamento. As proteínas *MIKC* agruparam-se em 13 subfamílias. As proteínas MADS-box *MIKC** foram utilizadas como um grupo externo. Adaptado de DÍAZ-RIQUELME *et al.* (2009).

Com o objetivo de caracterizar a expressão do gene *VvAGL11*, MEJÍA *et al*. (2011), por meio de ensaios de PCR quantitativa precedida de transcrição reversa (*RT-qPCR,* do inglês, *reverse transcription-quantitative PCR),* observaram que transcritos de *VvAGL11* são 25 vezes mais abundantes no estádio de fruto em comparação com os estádios de prefloração e floração em genótipos de videira sem sementes. Outro dado interessante refere-se ao fato de que, no estádio de fruto, a expressão do gene é acentuada em genótipos homozigotos para o alelo normal de *VvAGL11*, mostrando uma expressão 25 vezes maior do que em genótipos homozigotos para o alelo mutado, enquanto que os genótipos heterozigotos demonstraram um nível intermediário da expressão do gene. O sequenciamento da região promotora e codificadora de *VvAGL11* realizado por estes autores não permitiu concluir se as variações alélicas identificadas são responsáveis pelo fenótipo de apirenia e não foram realizadas análises funcionais neste trabalho (MEJÍA *et al*., 2011).

Paralelamente a estes estudos, nosso grupo analisou uma progênie de videira gerada a partir do cruzamento de uma cultivar apirênica 'Crimson Seedless' com o híbrido complexo resistente ao míldio 'Villard Blanc'. Os resultados obtidos a partir do mapa genético construído entre estas duas cultivares permitiram confirmar o QTL para a ausência de sementes dentro do *locus SdI* na extremidade do cromossomo 18 e com LOD (do inglês, *Linked Open Data*) máximo associado ao marcador molecular microssatélite VMC7F2 (REVERS *et al*., 2010).

Na presente Dissertação de Mestrado, a hipótese de trabalho foi fundamentada na seguinte afirmação: a extrema diminuição da expressão de *VvAGL11* encontrada em genótipos apirênicos de videira é a causa do fenótipo de apirenia, uma vez que o gene é essencial para a morfogênese da semente em uvas.

2 OBJETIVOS

2.1 OBJETIVO GERAL

Caracterizar as estruturas do gene *VvAGL11* na cultivar pirênica Chardonnay e na cultivar apirênica Sultanina por meio do sequenciamento de suas variações alélicas e da exploração da sua expressão gênica diferencial, visando uma maior compreensão de seu papel durante a morfogênese das sementes em videira.

2.2 OBJETIVOS ESPECÍFICOS

- Caracterizar as variações alélicas do gene *VvAGL11* em videira apirênica e pirênica;

- Caracterizar o perfil transcricional do gene *VvAGL11* em diferentes estádios de desenvolvimento do fruto;

- Analisar morfologicamente a semente de 'Chardonnay' e o traço de semente de 'Sultanina';

- Determinar o padrão de expressão espaço-temporal de *VvAGL11* em videira apirênica e com semente durante o desenvolvimento reprodutivo.

3 CAPÍTULO I

THE *AGAMOUS-LIKE 11* TRANSCRIPTION IS ESSENTIAL FOR SEED MORPHOGENESIS IN GRAPEVINE

Manuscrito a ser submetido ao periódico 'Journal of Experimental Botany`

Title

The *AGAMOUS-LIKE 11* transcription is essential for seed morphogenesis in grapevine

Authors

Jaiana Malabarba[1,2,*], Vanessa Buffon[2,*], Jorge E. A. Mariath[3], Marcos Letaif Gaeta[3]

Marcelo C. Dornelas[4], Márcia Margis-Pinheiro[1], Giancarlo Pasquali[1], Luís F. Revers[2,‡]

[1]Graduate Program in Cell and Molecular Biology, Centro de Biotecnologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, 91501-970, Brazil

[2]Laboratory of Plant Molecular Genetics, Centro Nacional de Pesquisa de Uva e Vinho, Empresa Brasileira de Pesquisa Agropecuária, Bento Gonçalves, RS, 95700-000, Brazil

[3]Laboratory of Vegetal Anatomy, Centro de Botânica, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, 91501-970, Brazil

[4]Graduate Program in Vegetal Biology, Universidade Federal de Campinas, Campinas, São Paulo, 13083-970, Brazil

[*]These authors contributed equally to this work.

† Current address: Laboratory of Plant Molecular Genetics, Centro Nacional de Pesquisa de Uva e Vinho, Empresa Brasileira de Pesquisa Agropecuária, Rua Livramento, 515, P.O. Box 130, CEP: 95700-000, Bento Gonçalves, RS, Brazil

‡ To whom correspondence should be addressed. E-mail: luis.revers@embrapa.br

Tel: +55 54 3455 8034

Number of figures: 8
Number of supplementary tables: 2
Number of supplementary figures: 3

Footnotes:

The nucleotide sequences reported in this paper have been submitted to GenBank under the accession numbers KM401845, KM401846, KM401847 and KM401848.

Abstract

Despite the wide and extensive appreciation of seedless grapes, little is known about the mechanisms that drive apireny. *VvAGL11* is a candidate gene involved in *Vitis vinifera* seed morphogenesis and studies showed that its expression is mainly localized in fruits of seeded grapes, while seedless varieties exhibits significantly lower levels of transcripts. Grapevine *AGL11* is a MADS-box gene, possibly orthologous to *Arabidopsis AGL11*, with an important role in ovule development. Our study aimed to characterize *VvAGL11* allelic variations in a seeded (Chardonnay) and in a seedless (Sultanina) grapevine cultivars, allowing us to observe several mutations that might be responsible for gene's loss of function. *VvAGL11* transcript levels corresponding to each phenotype were evaluated by RT-qPCR during grapevine flower and fruit development. *VvAGL11* relative expression was significantly higher in seeds of 2, 4 and 6 weeks after fruit-set of the seeded variety whereas in the seedless grape its transcription was extremely low in all stages analyzed. We further characterized the patterns of *VvAGL11* spatial and temporal expression via *in situ* hybridization. Transcript accumulation was specifically detected in the dual medium integument layer of the seeds, which is responsible for the elongation of seed coat and necessary to determine final seed size. Nevertheless, in seedless grape, no hybridization signals were visible and a morphological analysis showed an apparent loss of identity of the medium integument layer of the seed traces. Our data provide insights of the important role of *VvAGL11* in seed morphogenesis suggesting that depletion of its expression may be the main responsible for the erroneous development of a highly essential seed layer therefore culminating in the typical apirenic phenotype.

Keywords: apireny, grapevine, *in situ* hybridization, seed, Sultanina, *VvAGL11*.

Introduction

Seedlessness is one of the most appreciated features in table grapes along with enlarged fruit size, both being main goals in breeding programs (Varoquaux *et al*., 2000; Cabezas *et al*., 2006; Lazzarotto and Fioravanço, 2012). In the past two decades, the worldwide market of *in natura* grapes increased about 26% per year, while the production of such grapes grew at a 13% annual rate (OIV, 2012; FAO, 2012). In grapevine, the absence of seeds, also called apireny, is divided in two biological processes. One is known as parthenocarpy, when fecundation does not occur and there is no seed formation, as in raisins. The second process is called stenospermocarpy, in which fertilization takes place to form the fruit but it is followed by embryo abortion due to the cease of endosperm development, normally after four weeks of fruit growth. The consequence of stenospermocarpy is the production of berries with reduced seed size, known as seed traces (Bouquet and Danglot, 1996; Mejía, 2011).

Several studies have shown that the presence of a dominant allele in the region of the *Seed Development Inhibitor* (*SdI*) locus, located in *Vitis vinifera* distal portion of chromosome 18, is responsible for 50 to 90% of the total phenotypic variance of seedleessness (Bouquet and Danglot, 1996; Lahogue *et al*., 1998; Cabezas *et al*., 2006; Costantini *et al*., 2008; Doligez *et al*., 2002; Mejía *et al*., 2007). *In silico* analysis allowed the identification of a gene, named *Vitis vinifera AGAMOUS-LIKE 11* (*VvAGL11*), that mapped to the same locus contig *SdI* (Constantini *et al*., 2008). The same work has also co-located *VvAGL11* with the molecular microsatellite marker VMC7F2, which was known to be a highly polymorphic marker capable of an efficiency of 95% in the detection of seedlessness in grapes (Constantini *et al*., 2008).

The *VvAGL11* gene (Vv18s0041g01880; Boss *et al*., 2002) was proposed as a strong candidate gene involved in the absence of seeds in grapevine (Costantini *et al*., 2008; Mejía *et al*., 2011; Revers *et al*., 2010). This MADS-box gene was shown to be a possible ortholog of *AGL11* (*STK*) of *Arabidopsis thaliana*, which acts on the identity and control of ovule and seed development (Favaro *et al*., 2003; Pinyopich *et al*., 2003; Díaz-Riquelme *et al*., 2009). *AtAGL11* mutant (*stk*) presents reduced number and size of seeds (Pinyopich *et al*., 2003; Brambilla *et al*., 2007).

The transcription factors of the MADS-box family have extremely important roles in the development of plants. They are responsible for controlling the identity of floral organs (Smaczniak *et al.*, 2012). In a study covering the MADS-box family in grapevine, Díaz-Riquelme *et al.* (2009) demonstrated that *VvAGL11* is induced in floral and fruit tissues, whereas it is repressed in roots, branches, leaves, buds and tendrils. More recently, another study showed that *VvAGL11* is 25 times more expressed at the stage of fruit compared to flower stages in genotypes homozygous for seedless in grapes. In heterozygous genotypes, an intermediate level of *VvAGL11* expression was observed (Mejía *et al.*, 2011).

We therefore hypothesized that *VvAGL11* might be responsible for controlling seed morphogenesis in grapevine since the decrease of its expression would cause the apirenic phenotype. In this work we report the identification of differences between *VvAGL11* allele sequences in apirenic and pirenic grapevines, observing several mutations in intragenic regions and a set of *SNPs* in the coding region of the gene exclusively in the apirenic genotypic background. We characterized the *VvAGL11* transcript levels, observing a 65-fold increase in expression in grapevine seeds compared to apirenic grapevine fruits. We also described its spatial and temporal expression patterns in reproductive tissues by *in situ* hybridization that allowed us to establish a correlation between the development of a specific seed layer with normal or decreased *VvAGL11* expression.

Materials and methods

Plant material

All samples were harvested from grapevines located at Embrapa Grape and Wine experimental areas in Bento Gonçalves, Rio Grande do Sul, Brazil (29° 09' 48" S, 51° 31' 42" O and 616 m altitude). The phenological scale followed was the one described by Baggiolini (1952; Supplementary Table 2). 'Chardonnay' was chosen for comparison with 'Sultanina' because of its similar phenological development. For *VvAGL11* transcriptional profile, 'Chardonnay' (seeded) and 'Sultanina' (seedless) organ and tissue samples were harvested at the stages of preanthesis flower, fruit set and fruits at 2, 4 and 6 weeks after fruit set in 2010/2011. For *in situ* hybridization and morphological assays, 'Chardonnay' and 'Sultanina' samples were harvested at H1, preanthesis flower, fruit set and fruits at 2, 4 and

6 weeks after fruit set in 2012/2013 harvest. Both cultivars were graffted on Paulsen 1103 rootstock.

Sequencing

Twenty-two sets of primers were designed to amplify sequences in the promoter and in exon/intron regions of *VvAGL11* (Supplementary Table 1) in order to sequence the whole gene. Each region exhibited a 300-500 bp overlapping sequence. PCR amplifications were performed with 10 to 25 ng of grapevine genomic DNA using Platinum® Pfx DNA polymerase (Applied Biosystems) as recommended by the manufacturer. RACE amplifications were done with the SMARTer™ RACE cDNA Amplification Kit (Clontech). PCR products were cloned into pGEM®-T Easy Vector (Promega) following the manufacturer instructions. Plasmid DNA was extracted by the alkaline lysis miniprep protocol modified by a CIA step (Maniatis, Fritsch and Sambrook, 1987). PCR products were treated with the Illustra ExoProStar reagent (GE Healthcare) as recommended by the manufacturer, and were sequenced in an ABI Prism® 310 Genetic Analyser (Applied Biosystems) using standard sequencing protocols described by Falavigna *et al.,* 2014.

Identification of Putative CArG Sequences

Genomic regions located 3 kb upstream of the *VvAGL11* ATG start codon, 377 bp downstream of the stop codon, and exons and introns were analyzed to identify CArG-Box sequences with the New Place bioinformatic program available at the Sogo website (http://sogo.dna.affrc.go.jp).

RNA extraction

Total RNA samples were extracted from frozen material (approximately 200 mg) by LiCl precipitation using the Zeng and Yang (2002) protocol modified by a purification scale adapted to 2 mL microcentrifuge tubes. Each extraction was conducted using three tubes that had their volumes pooled before the LiCl precipitation step. Genomic DNA in total RNA samples was removed using the TURBO DNA-free Kit (Ambion) according to the manufacturer's protocol. RNA integrity and quantity was monitored by agarose gel electrophoresis and spectrophotometric quantitation, respectively.

RT-qPCR analyses

Complementary DNAs were synthesized using the GeneAmp RNA PCR Core Kit (Applied Biosystems) according to manufacturer's instructions. Gene-specific primer pairs were designed for the two forms of the candidate gene (*VvAGL11F* 5'-CACTTAATGGGTGAT TCCTTGGC-3', *VvAGL11R* 5'-AGCAACTCATGCTTCTTCGACC-3'; and *VvAGL3F* 5'-ATTGTTCATCTGGGCATTTCG-3', *VvAGL3R* 5'-GGAGATGAAGTTGGCGGATA-3') and evaluated by Oligo Analyzer (IDT, http://www.idtdna.com). RT-qPCR was performed in a StepOnePlus Real-Time PCR System (Applied Biosystems). SYBR Green (Invitrogen) was used to monitor dsDNA synthesis and ROX (Invitrogen) was employed as passive fluorescence reference. Each biological sample was analyzed in technical triplicates. Cycling protocol consisted of one step at 95 °C for 10 min followed by 40 cycles of 95 °C for 15 s, 60 °C for 1 min, and finished by a dissociation curve between 60 °C and 95 °C. The specificity of PCR amplifications was assessed by the presence of a single peak in melting curves and by the visualization of single amplification products of expected size in 1% ethidium bromide gel electrophoresis. Primer efficiency was calculated by LinRegPCR (version 11.0, Ruijter *et al.,* 2009). Mean relative gene expression was calculated by the Pfaffl (2001) method with the *actin* gene (GenBank EC969944) as reference (Reid *et al*., 2006). Statistical analysis was performed using Prism 5.1 with One-way ANOVA and Tukey test.

Seed morphological assay

Seeds and seed traces were transferred into a fixation solution under vacuum (McDowell and Trump, 1976). Thereafter, samples were dehydrated in an increasing gradient of ethanol as described by Gabriel (1982), and embedded in 2-hydroxyethyl methacrylate resin according to Gerrits and Smid (1983). The 5 µm sections were obtained by a Leica RM 2255 microtome. The metachromatic reagent Toluidine Blue O (Feder and O'Brien, 1968) was used to determine seed structure. For the staining procedure, slides with sections were placed on a hot plate at 45 °C, and the reagent was added. After 15 min, slides were rinsed with 95% ethanol and dried at room temperature.

In situ hybridization analysis

'Chardonnay' and 'Sultanina' samples were fixed in 4% formaldehyde for 16 hours. To submerge the samples, vacuum was applied (approximately 600 mmHg) for 20 min. The material was stored under refrigeration (4 to 10 °C) after fixation. Thereafter, the tissues were dehydrated in ethanolyc series and embedded in paraffin. Longitudinal and transversal sections (8-10 μm) were prepared with a rotary microtome and mounted on silanized microscope slides. Gene-specific sense and antisense probes were designed at 3'UTR region (*VvAGL3F* 5'-ATTGTTCATCTGGGCATTTCG-3', *VvAGL3R* 5'-GGAGATGAAGTTGGCGGATA-3') corresponding to 185 nucleotides of the 3' UTR of *VvAGL11* gene. Probes were generated by digoxigenin (DIG)-labeling using T7 or SP6 RNA polymerase of the DIG RNA Labeling Kit (Roche). After the detection of the hybridization signals by immunostaining, slides were washed, dehydrated, and mounted using Entellan® (Merck).

Results

Sequencing of VvAGL11

To further characterize the structural organization of the *VvAGL11* gene, allelic variations were PCR amplified with specific primers from genomic DNA extracted from an apirenic grapevine, 'Sultanina', and a pirenic grapevine, 'Chardonnay' as previously described. A total of six PCR fragments were assembled, formed by overlapping primers sets showed in figure 1. The gene sequence is composed by the promoter, coding and intragenic regions (8 exons and 7 introns). After allele specific isolation and sequencing, we were allowed to identify two alleles for each cultivar. Because 'Chardonnay' is a direct offspring of Pinot Noir, the comparison with the 'Pinot Noir' (PN40024) sequence in the publicly available genome base was possible (http://genomes.cribi.unipd.it/gb2/gbrowse/public/*Vitis*_vinifera/).

The two *VvAGL11* alleles of 'Chardonnay' showed 99% sequence identity with the corresponding 'Pinot Noir' genome sequence for *VvAGL11*. One allele from 'Sultanina' also exhibited 99% identity with PN40024. However the other 'Sultanina' *VvAGL11* allele, the one with the molecular microsatellite marker VMC7F2 described with shorter length (198 bp; Constantini *et al*., 2008), presented a group of polymorphisms that differed this allele

from the others, and due to that it was named the mutant allele (Sultanina *mut*), as shown in Fig. 1. The group of polymorphisms was composed by 28 INDELs and 105 SNPs.

Among the observed alterations in *Sultanina mutant VvAGL11 allele*, two SNPs give rise to two amino acid substitutions (R590L and T628A) as shown in Fig. 2. A sequence alignment of the four *VvAGL11* alleles, PN40024, and the newest sequence of *VvAGL11* from Sultanina (Di Genova *et al*., 2014) is presented in Supplementary Fig. 1. With high fidelity amplication and sequencing, we were confident to define all VvAGL11 alleles' modifications. We also searched for CArG-box sequences, perfect CArG-boxes and CArG sequences with one mismatch deduced by a probability matrix. These *cis* elements are known to be the sites of MADS-box protein complex binding (Smaczniak *et al*., 2012). We identified ten putative CArG sequences in *VvAGL11*. One of them is located in the promoter region, eight in the second intron and one in the 3'-UTR region. The seventh CArG-box putative sequence of 'Sultanina' *VvAGL11 mut* has a nucleotide modification (Supplementary Fig. 1, black arrow). Sequence modification in these *cis* elements could disrupt *VvAGL11* transcription activation.

VvAGL11 expression isoforms

The 3'- and 5'-RACE experiments allowed us to visualize the mRNA isoforms of *VvAGL11* in apirenic and pirenic grapevines. Two products of amplification differing in size were obtained from 'Chardonnay' and one product was obtained from 'Sultanina'. After sequencing them, it was possible to confirm the specificity of the amplicons and to characterize each transcript form present in the cultivars.

The results revealed that 'Chardonnay' has two isoforms of *VvAGL11* mRNA. One isoform, named CH-1, is longer with 1,454 bp, being 732 bp of coding region. This alternative form increases the coding region in 60 nucleotides. The second 'Chardonnay' allele, named CH-2, has 958 bp and a coding sequence of 672 pb. 'Sultanina' also has two transcript forms, both with the same length (958 bp) and coding sequence (672 bp). The dissemblance of the two *VvAGL11* isoforms derived from 'Sultanina' is the ten SNPs found in the mutant allele (SU *mut*). The coding regions of CH-2 and 'Sultanina' allele 1 are identical to the *VvAGL11* gene predicted in the Pinot Noir reference genome. However, none of the Sultanina isoforms are completely identical to the 'Pinot Noir' gene since their 5'-UTR are different in size, as shown in Fig. 3.

Transcription profile of VvAGL11

Aiming to evaluate the expression of *VvAGL11* and its isoforms, we performed RT-qPCR analysis with mRNA samples extracted from the reproductive organs of 'Chardonnay' and 'Sultanina' at critical developing stages. Besides a pair of primers designed for regions of the actin reference gene, we employed two sets of primers in RT-qPCR studies to differentiate *VvAGL11* mRNA isoforms. The first *VvAGL11* primer set was composed of a forward primer designed to anneal in the last intronic region and a reverse primer that annealed in the 3-'UTR , therefore allowing the amplification of the longer CDS isoform (CH1). The second pair of primers was designed to anneal in the first (forward) and in the second (reverse) exons that allowed us to amplify simultaneously the two isoforms of *VvAGL11* (the shorter (CH1) and longer (CH2) CDS). Considering that in 'Sultanina' the isoforms differ in point mutations (10 SNPs) only, we employed the primer set flanking the first and second exons for the analysis of both isoforms (SU1 and SU *mut*).

The reproductive tissues evaluated were divided into initial and advanced developmental stages. The initial stages of development were represented by flowers at preanthesis and fruit-set. The advanced stages were represented by fruits at 2, 4 and 6 weeks of development after fruit establishment (fruit-set). Seeds were separated from the pulp in 'Chardonnay' berry samples and the whole berry was evaluated in 'Sultanina' samples. The amplicons were sequenced for determination of their specificity. Actin gene *VvACT* (Gen Bank EC969944) was used as reference.

Our RT-qPCR results showed that *VvAGL11* relative expression was similarly low at all stages sampled from the 'Sultanina' variety for both isoforms SU1 and SU *mut*. 'Chardonnay' flowers and berry pulps also demonstrated low transcript levels of *VvAGL11* for both isoforms CH1 and CH2. RT-qPCR with primers designed to the intronic region was also performed with 'Sultanina' mRNA samples and a minor level of expression was detected, probable descendent from residual from pre-mRNA during cDNA synthesis. Nevertheless, seeds of 'Chardonnay' from fruits at 2, 4 and 6 weeks after fruit set exhibited much higher accumulation of *VvAGL11* CH2 transcripts, an increase 15-fold higher than CH2 transcripts in berries at fruit set and 65-fold higher than 'Sultanina' whole berries. 'Chardonnay' CH1 allele relative expression was unstable through seed stages, fluctuating from 5 to 11 fold higher than fruit-set (Fig. 4).

Morphological analysis of seeds from apirenic and pirenic grapevines

V. vinifera seeds possess a particular shape resembling a pear, being triangular in cross sections (Fig. 5). The seeds are composed of a cuticle, an epidermis, two layers corresponding to the outer integument and the medium integument, both forming the seed coat at the ripening stage, and yet three inner integument layers that are positioned around the endosperm and embryo (Ribereau-Gayon and Peynaud, 1980). The structure of the seeds from 'Chardonnay' and seed traces from 'Sultanina' were found to be very different in shape, size and hardness (Fig. 5). For that reason, a morphological analysis was conducted to better characterize the development of endosperm, embryo and seed layers of both cultivars at 2, 4 and 6 weeks of fruit development. Descriptions of the structures as they evolve, examined with the metachromatic reagent toluidine blue O staining, are presented below and in Fig. 6.

Endosperm and Embryo. A normal and complete development of the endosperm and the embryo was observed in 'Chardonnay' seeds after 6 weeks of fruit development. 'Sultanina' seed traces showed an unevolved endosperm and no embryo could be distinguished at that stage.

Outer Integument. Parenchyma cells are the main constituent of the outer integument and cell layers are thicker in the ventral face of the seed. At the time of ripening there is an increase in parenchyma cell volume in 'Chardonnay', but the number of cells do not seem to change (Fig. 6A, 6C and 6E). In 'Sultanina' seed traces, no differences were observed in this layer, since there is no visible evolution of the seeds in this variety (Fig. 6B, 6D and 6F). Along with the outer integument, the epidermis covers the outer integument in both varieties.

Medium Integument. After 2 weeks of fruit development, one layer of rectangular palisade cells was observed in the seeds of both varieties (Fig. 6A and 6B). After 4 weeks, two layers of this integument were observed in 'Chardonnay' seeds (Fig. 6C), but this duplication was not observed in 'Sultanina' seed traces (Fig. 6D). After 6 weeks of fruit development, 'Chardonnay' seeds showed a sclerified and thicker medium integument (Fig. 6E) opposed to 'Sultanina' seed traces that exhibited the same structures of the initial stage of development.

Inner Integument. Three layers of inner integument were observed in the seeds at the initial stage of fruit development in both varieties (Fig. 6A and 6B), although only two layers

were present in later stages in 'Chardonnay' (Fig. 6C and 6E) and in 'Sultanina' (Fig. 6D and 6F).

Spatial and temporal accumulation of VvAGL11 transcripts

In situ hybridization (ISH) of the reproductive tissues of both grapevine varieties were performed in order to better characterize *VvAGL11* spatial and temporal expression patterns at the level of transcript accumulation. The stages of development analyzed were those described by Baggiolini (1952; Supplementary Table 2) including flower formation (H1), preanthesis (PA), fruit-set (FS), seeds at 2, 4 and 6 weeks after flower fertilization.

After ISH with the complementary (antisense) probe, no expression of *VvAGL11* was detected at the stages H1 (Fig. 7B), PA (Fig. 7C), FS (Fig. 7D) and at 6 weeks of fruit development (Fig. 7L) in 'Chardonnay'. *VvAGL11* transcripts exhibited a high accumulation in seeds after 2 (Fig. 7E and 7F) and 4 weeks (Fig. 7G to 7J) of development in 'Chardonnay'. The ISH signal was clearly visible in the medium integument layer of the seeds (Fig. 7K). In 'Sultanina', no gene expression was observed at any reproductive tissue/organ or stage of development (Fig. 8A to 8F). Important to mention, the ISH slides with the antisense probe were always compared with slides hybridized with the sense *VvAGL11* probe as control in the analysis of 'Chardonnay' and 'Sultanina' (Supplementary Fig. 2 and 3, respectively).

Discussion

Spontaneous somatic variants have been selected all the way through grapevine domestication, hence creating new and different cultivars with interesting traits such as berry size, taste, color, and seedlessness (This *et al.,* 2006). Grapevine somatic variants have also been useful tools for studies of gene function, whereas they result from single mutation events in a given genetic background (Fernandez *et al.,* 2010). *VvAGL11* was proposed as a candidate gene involved in the absence of seeds (Costantini *et al*., 2008; Revers *et al*., 2010; Mejía *et al*., 2011). With the attempt to better evidence the essentiality of *VvAGL11* in grapevine apireny, we described here *VvAGL11* gene structure in apirenic ('Sultanina') and pirenic ('Chardonnay') grapevines, its transcript isoforms and profiles of accumulation, and

its spatial-temporal expression pattern during reproductive organ/tissue development and seed morphogenesis.

By means of a PCR strategy based on a high fidelity polymerase, sequencing of *VvAGL11* allelic variants from 'Sultanina' and 'Chardonnay' was performed, allowing the identification of a group of polymorphisms (Fig. 1). No transposon or large sequence insertion or deletion was found in 'Sultanina' that could explain the gene loss of transcription observed in previous data (Mejía *et al.,* 2011). Sequences were compared with the grapevine reference ('Pinot Noir') genome and to a recent work that reported the whole genome sequence of 'Sultanina', producing a catalog of structural variants for grapevine (Di Genova *et al.,* 2014). We found two amino acid modifications in 'Sultanina's' *VvAGL11* coding region (Fig. 2). Arginine and threonine were substituted by two more hydrophobic amino acids, leucine and alanine, respectively. These amino acid substitutions were localized in the C-terminal portion of the VvAGL11 protein, a well known region responsible for activity and ternary complex formation (Egea-Cortines *et al.,* 1999). Furthermore, 17 INDELs and 53 additional SNPs were identified in the second intron of the 'Sultanina' mutant allele (Supplementary Fig. 1). The second intron of MADS-box AG subfamily members is known to present a quite large size (*AtAG* ~3 kb, *AtAGL11* ~1,8 kb, and *VvAGL11* ~4 kb) and to be a region with important *cis* elements that may control tissue specific expression of AG and AG-like genes (Deyholos, 2000; Hong *et al*., 2003). Interestingly, MADS-box proteins form complexes of high order, often in tetrameric junctions made by two dimers that bind to two DNA consensus sequences called CArG-box [CC(A/T)$_6$GG] separated by up to 300 bp (Liu *et al*., 2008). A CArG-box modification was observed in 'Sultanina' *mut* allele, in the seventh putative sequence. This *cis* element is only 84 bp distant from another CArG-box what would suggest their relevance in gene regulation.

We performed the first analysis of *VvAGL11* transcript isoforms and compared them to the genome gene-predicted models (Fig. 3). The transcription profile of *VvAGL11* isoforms provided complete data for each expression isoform during the development of flowers, fruits and seeds. 'Sultanina' alleles presented a low level of expression in all organs and stages of development, including berries, while the 'Chardonnay' alleles were expressed at very low levels in the pulp, but highly expressed in seeds (Fig. 4). Of the two 'Chardonnay' alleles, one possesses a larger coding region, able to encode 20 additional amino acids (CH1). The sequence of CH1 transcripts was also found in a 'Cabernet Sauvignon' cDNA

library derived from berries (GenBank CB974197.1). This could cause conformational changes in the C-terminal portion of VvAGL11 protein, yet no abnormal seed morphogenesis is observed in Chardonnay neither in Cabernet Sauvignon cultivars. Thus it is clear that this 60 nucleotides may not be relevant to seed phenotype, therefore the biological relevance of these 20 additional amino acids in the C-terminal VvAGL11 protein remains to be elucidated. The relative expression of CH1 allele in seeds showed a fluctuation between 5 to 11-fold compared to fruit-set (Fig. 4). This instability was possibly due to the random amplification of *VvAGL11* pre-mRNA since primers employed allowed the amplification of intronic regions. Taken together, our results suggested that *VvAGL11* has indeed a major role in seed development. Without its expression (transcript accumulation), there is no normal seed formation.

The interaction of proteins encoded by MADS-box genes of classes A, B, C, D, and E forms the model of floral quartets (Theissen and Saedler, 2001; Dornelas and Dornelas, 2005). The AGAMOUS subfamily of *MADS-box* genes is known to have redundant functions in *A. thaliana* since *AtAGL11* is the major responsible gene in the regulation of ovule development along with other two genes of class D, *SHATERPROOF1* and *SHATTERPROOF2* (Colombo *et al*., 1995; Pinyopich *et al.,* 2003). The *VvAGL11* gene was proposed as a possible ortholog of *AtAGL11*, however no gene has been identified yet as orthologous to *SHP1* or *SHP2* in grapevine (Díaz-Riquelme *et al*., 2009). These data suggest that *VvAGL11* might be the only class D gene in grapevine and a possible model of floral quartet would be composed by *VvAG1* and *VvAG2* (*AGAMOUS* genes), and one of the *SEPALLATA* genes of grapevine (*VvSEP1*, *VvSEP2, VvSEP3*, *and VvSEP4)* as previously described by Boss et al. (2002) and Díaz-Riquelme et al. (2008). Additional studies of the VvAGL11 protein are necessary to confirm this hypothesis.

Previous studies have described grapevine's seed anatomy (Pratt, 1971; Ribereau-Gayon and Peynaud, 1980) as well as the changes of internal layers towards seed ripening (Cadot *et al*., 2006), but, as much as we are concerned, no study has demonstrated detailed morphological parameters in seeds of stenospermocarpy grapevines, specially seed traces. The main objective of our morphological assay was to compare normal seeds from 'Chardonnay' and seed traces from 'Sultanina' as they resemble so differently from each other (Fig. 5). Our analysis showed that the most substantial difference was at the medium integument of the seed coat, which did not develop normally in 'Sultanina' seed traces,

preserving its initial features until the complete maturation of the fruits. As expected, 'Chardonnay' seeds developed correctly with the elongation and duplication of the medium integument, along with normal endosperm growth (Fig. 6). The nutrient flux in seeds occurs in a centripetal way, from the outer integument to the inner, going through apoplasts to the endosperm and to the embryo (Stadler *et al.*, 2005). Garcia et al. (2005) have previously shown that there is a strong maternal effect of the integuments in the control and determination of final seed size, due to the elongation of these layers and, in case of elongation absence, there is a compensation effect promoted by the duplication of these layers. Seed traces formation are likely originated due to disruption of seed tissues normal development. Probably the erroneous formation of the medium integument seed layer that occurs in stenospermocarpic grapevines could interfere in seed nutrient intake and cause the degeneration of some tissues during seed development, like the endosperm.

ISH assays were performed for better characterization of *VvAGL11* spatial and temporal expression patterns, in 'Chardonnay' and 'Sultanina', during seed morphogenesis in order to identify its transcription specific tissue localization. At the time of ISH assays the transcription isoforms were not known and the probe used for hybridization was designed according to the *VvAGL11* gene predicted model (GSVIVT01025945001) available at http://www.genoscope.cns.fr. Therefore, the whole probe (185 bp) hybridizes with CH1 mRNA isoform while 38 nucleotides hybridizes with CH2, SU1 and SU *mut* mRNA isoforms. The transcripts accumulation were localized in the medium integument layer of 'Chardonnay' seeds of 2 and 4 weeks (Fig. 7), while no accumulation of transcripts was found in 'Sultanina' seed traces (Fig. 8). After 6 weeks of 'Chardonnay' seed development, no hybridization signals were detected (Fig. 7L), this is due probably because the medium integument layer has already started lignification process, once it will be part of the mature and hard seed coat (Haughn & Chaudhury, 2005). This data is in agreement with *VvAGL11* RT-qPCR transcriptional profile, where 'Sultanina' shows almost no expression in any of the stages evaluated. The particular accumulation of *VvAGL11* transcripts in a specific layer of the seed coat in Chardonnay cultivar, combined with the morphological differences in this same layer when comparing Chardonnay and Sultanina cultivars, suggest that this gene is essential for the elongation and duplication of the medium integument of the seed coat. Furthermore, seed growth is dictated by the coordinated development of endosperm and

integuments and, hence, a spatial and temporal coordination of endosperm and integument growth is critical for normal seed development (Garcia *et al.,* 2005).

Taken together, our results allow us to propose that the absence of *VvAGL11* expression, a MADS-box class D gene of grapevine, is responsible for the erroneous development of seeds. The extreme diminution of final seed size could be due to the non-differentiation of the medium integument seed layer, causing the cease of the elongation ans duplication of the seed coat. Within this loss of identity, the endosperm would have no normal development as well, stopping its growth and causing the death of the embryo leading to the formation of a seed trace.

Acknowledgments

References

Baggiolini M. 1952. Les stades repères dans le developpement annuel de la vigne et leur utilisation pratique. Revue Romande d'Agriculture, de Viticulture et d'Arboriculture 8, 4–6.

Berger F, Grini PE, Schnittger A. 2006. Endosperm: an integrator of seed growth and development. Current Opinion in Plant Biology 9, 664–670.

Boss PK, Sensi E, Hua C, Davies C, Thomas MR. 2002. Cloning and characterization of grapevine (*Vitis vinifera* L.) MADS-box genes expressed during inflorescence and berry development. Plant Science 162, 887–895.

Bouquet A, Danglot Y. 1996.Inheritance of seedlessness in grapevine (*Vitis vinifera* L.). Vitis 35, 35-42.

Brambilla V, Battaglia R, Colombo M, Masiero S, Bencivenga S, Kater MM, Colombo L. 2007. Genetic and molecular interactions between BELL1 and MADS box factors support. Genetic and molecular interactions between BELL1 and MADS box factors support ovule development in *Arabidopsis*. The Plant Cell 19 (8), 2544–2556.

Cabezas JA, Cervera MT, Ruiz-Garcia L, Carreno J, Martinez-Zapater JM. 2006. A genetic analysis of seed and berry weight in grapevine. Genome 49 (12), 1572–1585.

Cadot Y, Minãna-Castelló MT, Chevalier M. 2006. Anatomical, Histological, and Histochemical Changes in Grape Seeds from *Vitis vinifera* L. cv Cabernet franc during Fruit Development Agriculture and Food Chemistry 54, 9206-9215.

Colombo L, Franken J, Koetje E, Van Went J, Dons HJ, Angenent Gc, Van Tunen AJ. 1995. The petunia MADS box gene FBP11 determines ovule identity. The Plant Cell 7 (11), 1859-1868.

Costantini L, Battilana J, Lamaj F, Fanizza G, Grando MS. 2008. Berry and phenology-related traits in grapevine (*Vitis vinifera* L.): from quantitative trait loci to underlying genes. BMC Plant Biology 8 (38), 1471-2229.

Deyholos MK and Sieburth LE. 2000. Separable whorl-specific expression and negative regulation by enhancer elements within the AGAMOUS second intron. The Plant Cell 12, 1799–1810.

Di Genova A, Almeida, AM, Muñoz-Espinoza C, Vizoso P, Travisany D, Moraga C, Pinto M, Hinrichsen P, Orellana A, Maass A. 2014. Whole genome comparison between table and wine grapes reveals a comprehensive catalog of structural variants. BMC Plant Biology 7 (14), 1471-2229.

Díaz-Riquelme J, Lijavetzky D, Martínez-Zapater JM, Carmona MJ. 2009. Genome wide analysis of *MIKC*^C-type MADS-box genes in grapevine. Plant Physiology 149, 354–369.

Doligez A, Bouquet A, Danglot Y, Lahogue F, Riaz S, Meredith CP, Edwards KJ, This P. 2002. Genetic mapping of grapevine (*Vitis vinifera* L.) applied to the detection of QTLs for seedlessness and berry weight. Theoretical and Applied Genetics 105 (5), 780–795.

Dornelas MC and Dornelas O. 2005. From leaf to flower: revisiting Goethe's concepts on the ¨metamorphosis¨ of plants. Brazilian Journal of Plant Physiology 17 (4), 335-343.

Egea-Cortines M, Saedler H, Sommer H. 1999. Ternary complex formation between the MADS-box proteins SQUAMOSA, DEFICIENS and GLOBOSA is involved in the control of floral architecture in Antirrhinum majus. EMBO Journal 18, 5370–5379.

FAO, Food and Agriculture Organization of the United Nations. 2014. Available at <http://www.faostats.org/>.

Falavigna VS, Porto DD, Buffon V, Pinheiro-Margis M, Pasquali G, Revers LF. 2014. Differential Transcriptional Profiles of Dormancy-Related Genes in Apple Buds. Plant Molecular Biology Reporter 32 (4), 796-813.

Favaro R, Pinyopich A, Battaglia R, Kooiker M, Borghi L, Ditta G, Yanofsky MF, Kater M, Colombo L. 2003. MADS-Box Protein Complexes Control Carpel and Ovule Development in *Arabidopsis*. The Plant Cell 15, 2603-2611.

Feder N and O'brien TP. 1968. Plant Microtechnique, Some Principles and New Methods. American Journal of Botany 55, 123–142.

Fernandez L, Torregrosa L, Segura V, Bouquet A, Martinez-Zapater JM. 2010. Transposon-induced gene activation as a mechanism generating cluster shape somatic variation in grapevine. The Plant Journal, 545–557.

Fischer BM, Salakhutdinov I, Akkurt M, Eibach R, Edwards KJ, Töpfer R, Zyprian EM. 2004. Quantitative trait locus analysis of fungal disease resistance factors on a molecular map of grapevine. Theoretical and Applied Genetics 108, 501-515.

Gabriel BL. 1982. Biological electron microscopy. New York, Van Nostrand Reinhold Company. p. 264.

Garcia D, Fitz-Gerald JN, Berger F. 2005. Maternal control of integument cell elongation and zygotic control of endosperm growth are coordinated to determine seed size in *Arabidopsis*. The Plant Cell 17, 52–60.

Gerrits PO and Smid L. 1983. A new less toxic polymerization system for the embedding of soft tissue in glycol methacrylate and subsequent preparing of serial sections. Journal of Microscopy 132, 81-85.

Haughn G and Chaudhury A. 2005. Genetic analysis of seed coat development in *Arabidopsis*. Trends in Plant Science 10, 472-477.

Hong RlL Hamaguchi L, Busch MA, Weigel D. 2003. Regulatory elements of the floral homeotic gene AGAMOUS identified by phylogenetic footprinting and shadowing. The Plant Cell 15 (6), 1296-1309.

Lahogue F, This P, Bouquet A. 1998. Identification of a codominant marker linked to the seedlessness character in grapevine. Theoretical and Applied Genetics 97, 1638-1649.

Lazzarotto JJ and Fioravanço JC. 2012. Comércio exterior mundial e brasileiro de uva de mesa: análise de indicadores de competitividade, tendência e sazonalidades. Documentos Embrapa Uva e Vinho 44.

Lefort F and Douglas GC. 1999. An efficient micro-method of DNA isolation from mature leaves of four hardwood tree species Acer, Fraxinus, Prunus and Quercus. Annals of Forest Science 56, 259-263.

Liu C, Chen H, Er HL, Soo HM, Kumar PP, Han JH, Liou YC, Yu H. 2008. Direct interaction of AGL24 and SOC1 integrates flowering signals in *Arabidopsis*. Development 135, 1481-1491.

Mcdowell EM and Trump BR. 1976. Histological fixatives for diagnostic light and electron microscopy. Archives of Pathology & Laboratory Medicine 1000, 405-414.

Mejía N, Gebauer M, Munoz L, Hewstone N, Munoz C, Hinrichsen P. 2007. Identification of QTLs for seedlessness, berry size, and ripening date in a seedless x seedless table grape progeny. American Journal of Enology and Viticulture 58 (4), 499–507.

Mejía N, Soto B, Guerrero G, Casanueva X, Houel C, Miccono MLA, Ramos R, Le Cunff L, Boursiquo JM, Hinrichsen P, Adam-Blondo AF. 2011. Molecular, genetic and transcriptional evidence for a role of VvAGL11 in stenospermocarpic seedlessness in grapevine. BMC Plant Biology 11 (57), 1471-2229.

OIV. Organization Internacional de la Vigne et du Vin (OIV), 2014. Paris. Statiques du secteur vitivinicole mondial. Available at <http://www.oiv.int/oiv/cms/index>.

Pfaffl MW. 2001. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Research 29, 45.

Pinyopich A, Ditta Gs, Savidge B, Liljegren Sj, Baumann E, Wisman E, Yanofsky Mf. 2003**.** Assessing the redundancy of MADS-box genes during carpel and ovule development. Nature 424, 85–88.

Pratt C. 1971. Reproductive anatomy in cultivated grapes. A review. American Journal of Enology and Viticulture 22 (2), 92-109.

Reid KE, Olsson N, Schlosser J, Peng F, Tlund ST. 2006. An optimized grapevine RNA isolation procedure and statistical determination of reference genes for real-time RT-PCR during berry development. BMC Plant Biology 6, 27-37.

Revers LF, Welter LJ, Irala PB, Silva DC, Lampe VS, Oliveira PRD, Garrido LR. 2010. Co-Localization of QTLs for Seedlessness and Downy Mildew Resistance in Grapevine. In: International Conference on Grapevine Breeding and Genetics 10: 449-456.

Ribereau-Gayon J and Peynaud E. 1980. Sciences et techniques de la Vigne. Tome I. Biologie de la Vigne. Sols de Vignobles, Dunod: Paris, Vol. 1, 327.

Ruijter JM, Ramakers C, Hoogaars WM, Karlen Y, Bakker O, van den Hoff MJ, Moorman AF. 2009. Amplification efficiency: linking baseline and bias in the analysis of quantitative PCR data. Nucleic Acids Research 37 (6), e45.

Smaczniak C, Immink RGH, Angenent GC, Kaufmann K. 2012. Developmental and evolutionary diversity of plant MADS-domain factors: Insights from recent studies. Development 139, 3081–3098.

Stadler R, Lauterbach C, Sauer N. 2005. Cell-to-cell movement of green fluorescent protein reveals post-phloem transport in the outer integument and identifies symplastic domains in *Arabidopsis* seeds and embryos. Plant Physiology 139, 701–712.

Theissen G and Saedler H. 2001. Plant biology. Floral quartets. Nature 409, 469-471.

This P, Lacombe T, Thomas MR. 2006. Historical origins and genetic diversity of wine grapes. Trends in Genetics 22 (9), 511-519.

Varoquaux F, Blanvillain R, Delseny M, Gallois P. 2000. Less is better: new approaches for seedless fruit production. Trends in Biotechnology 18, 233–242.

Zeng Y and Yang T. 2002. RNA isolation from highly viscous samples rich in polyphenols and polysaccharides. Plant Molecular Biology Reports 20, 417a-417e.
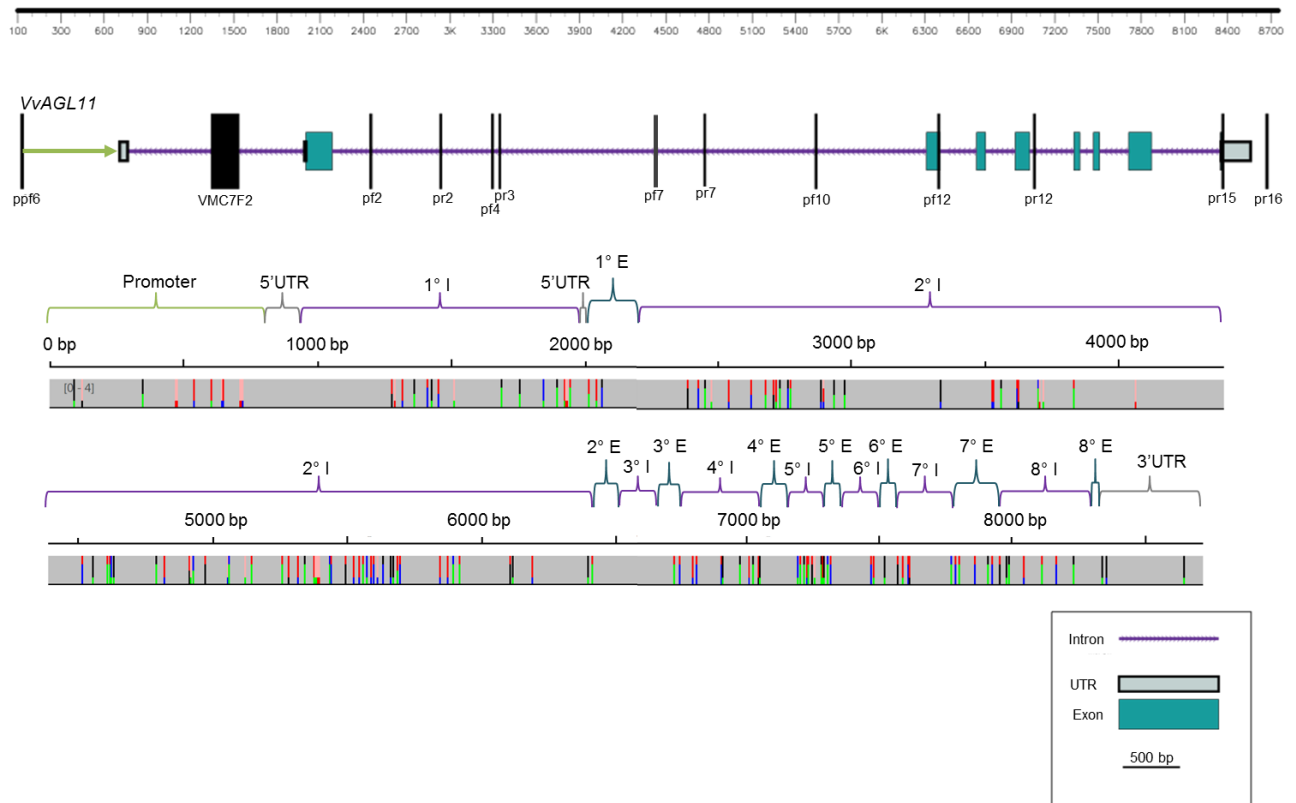
Fig. 1. Representative image of *VvAGL11*. DNA structure is composed by a promoter region, 5'-UTR, 8 introns, 8 exons and 3'-UTR. The black lines represents the localization of the primers used for allele amplification. Below, the alignment with the set of polymorphisms derived from 'Sultanina' and 'Chardonnay' *VvAGL11* alleles. Colors stripes represent SNPs (A-green, C-blue, T-red, G-black), and the salmon color indicates INDELs.
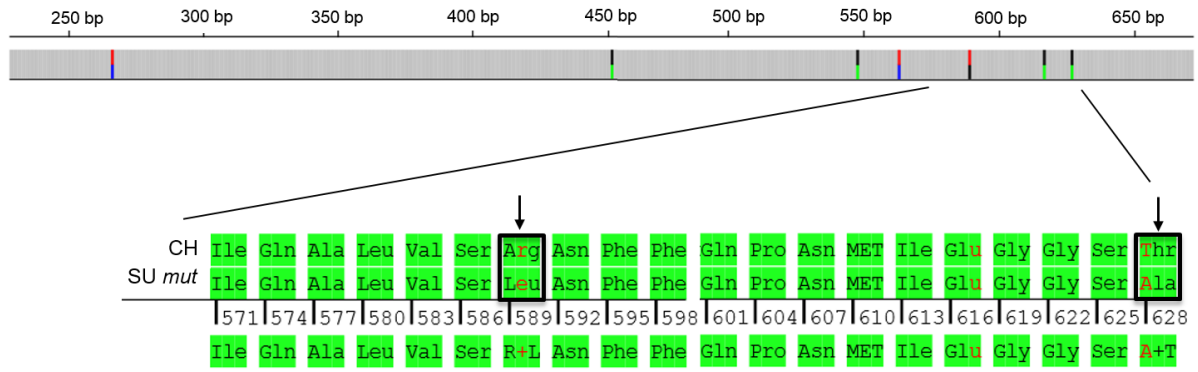
Fig. 2. Alignment of the coding regions of *VvAGL11* alleles where two amino acid modifications were observed. Alleles from 'Pinot Noir' (PN40024), 'Chardonnay' (both *VvAGL11* alleles) and 'Sultanina' normal and mutant allele. Colors stripes represent SNPs (A-green, C-blue, T-red, G-black).
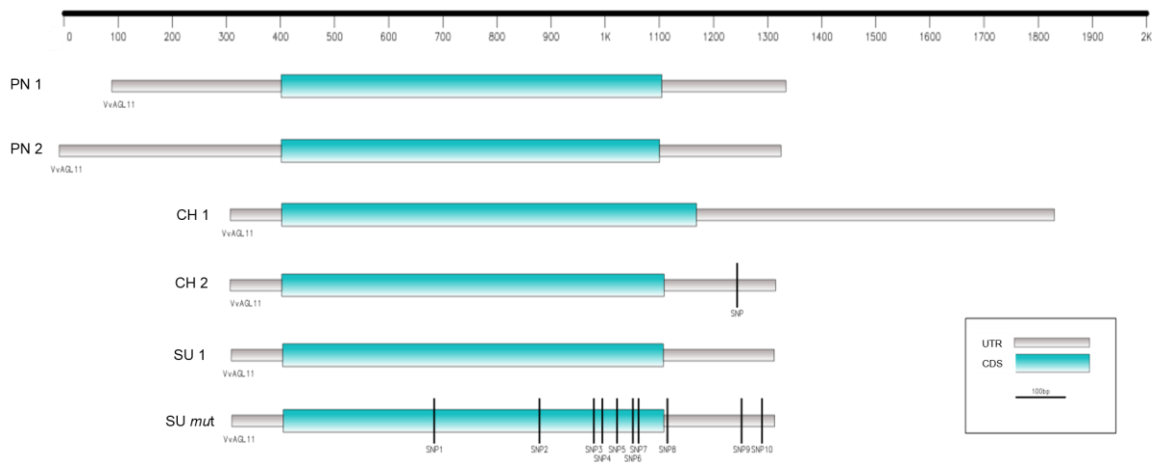
Fig. 3. Comparison between *VvAGL11* mRNA forms. PN 1 and PN 2 represent mRNA versions of *VvAGL11* from the Pinot Noir reference cultivar (VIT_218s0041g01880.1 and VIT_218s0041g01880.2, respectively). 'Chardonnay' allele 1 (CH1), 'Chardonnay' allele 2 (CH2), 'Sultanina' allele 1 (SU1) are compared with the 'Sultanina' mutant allele (SU *mut*). SNPs represented by black stripes (GenBank KM401845, KM401846, KM401847 and KM401848).
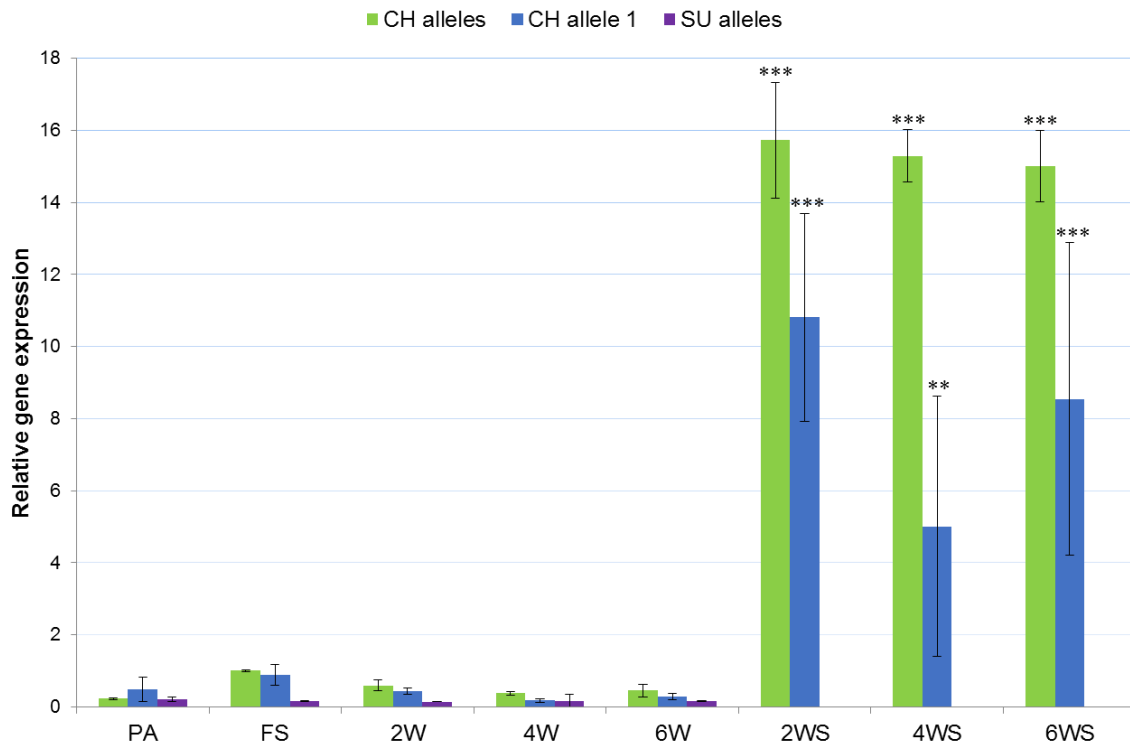
Fig. 4. Relative gene expression of *VvAGL11* alleles from 'Chardonnay' and 'Sultanina' via RT-qPCR. The developmental stages sampled are shown in the x-axis: **(PA)** pre anthesis flower; **(FS)** fruit set; **(2W-4W-6W)** fruits at 2, 4 and 6 weeks after fruit set and 'Chardonnay' seed stages **(2WS-4WS-6WS)**. 'Chardonnay' fruit-set was used as reference. The relative expression expressed in the y-axis was calculated by the Pfaffl (2001) method. Stander deviation is showed for each stage and sample. Asterisks indicate statistical significance between 'Chardonnay' alleles in seed stages to others stages evaluated for the 'Chardonnay' and 'Sultanina' alleles (Tukey test: ** $p<0.01$, *** $p<0.001$).
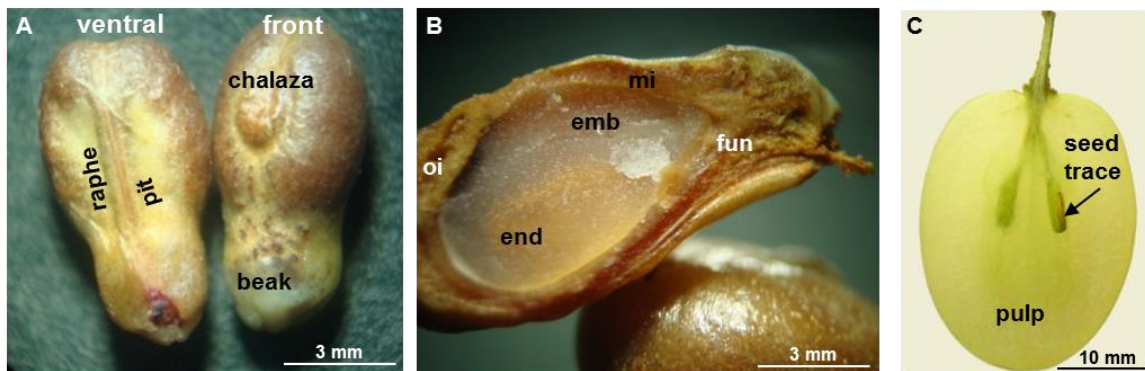
Fig. 5. Seeds and seed traces in *V. vinifera*. (A) Mature 'Chardonnay' (CH) seeds at their ventral and frontal view. Main structures are indicated. (B) CH seed cross section, with the internal view exhibiting outer integument (oi), medium integument (mi), endosperm (end), embryo (emb), and funiculus (fun). (C) Mature 'Sultanina' (SU) berry showing its seed traces.
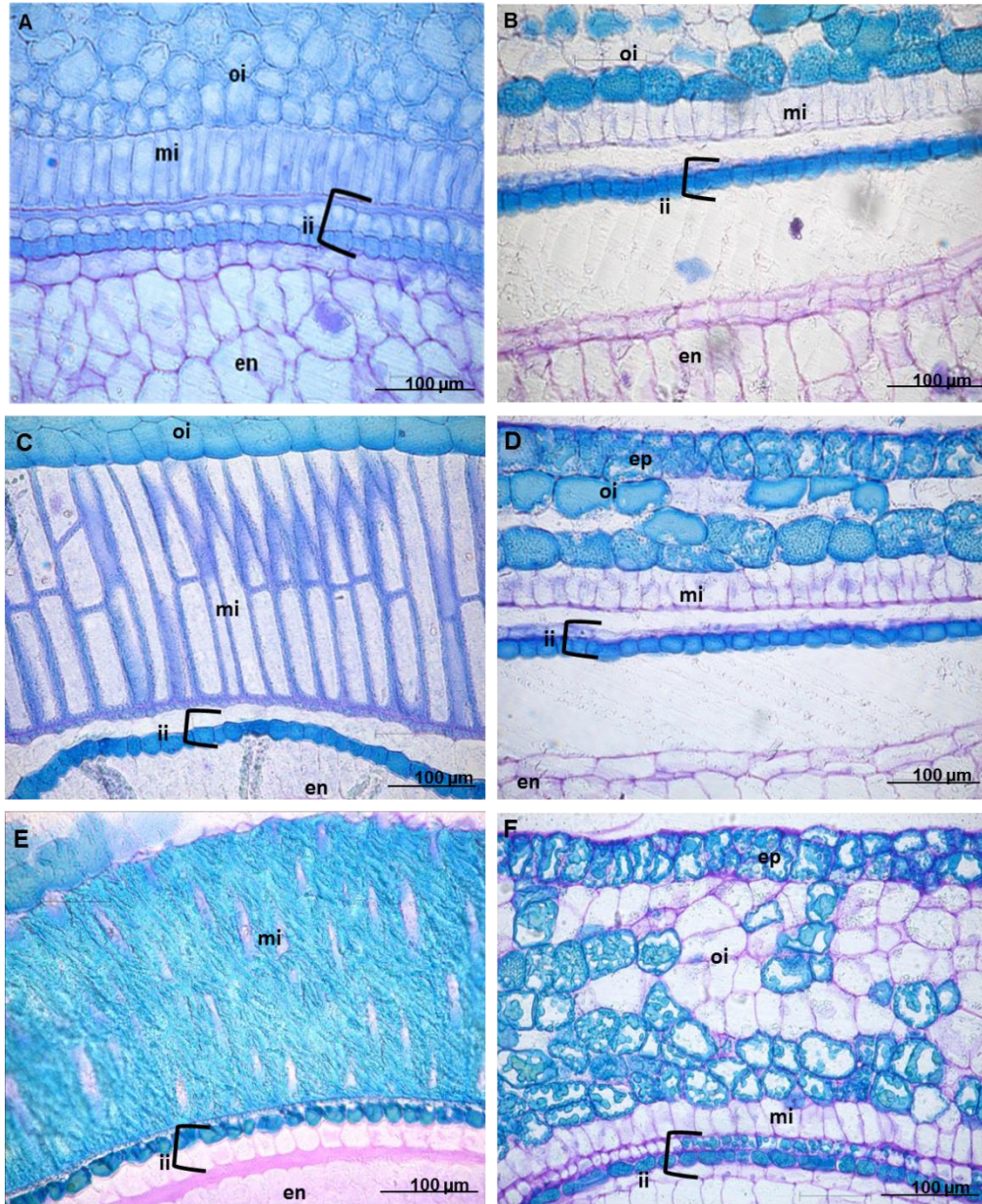
Fig. 6. Grapevine seed structure development from 2 to 6 weeks after fruit set. Light micrographs of grape seed cross sections were stained with toluidine blue O. The cross sections in the median area of the seeds of 'Chardonnay' and seed traces of 'Sultanina' enable the visualization of three defined integuments. 'Chardonnay' seed: 2 weeks **(A)**, 4 weeks **(C)** and 6 weeks **(E)**. 'Sultanina' seed trace: 2 weeks **(B)**, 4 weeks **(D)** and 6 weeks **(F)**. Endosperm (en), epidermis (ep), internal integument (ii), medium integument (mi), outer integument (oi).
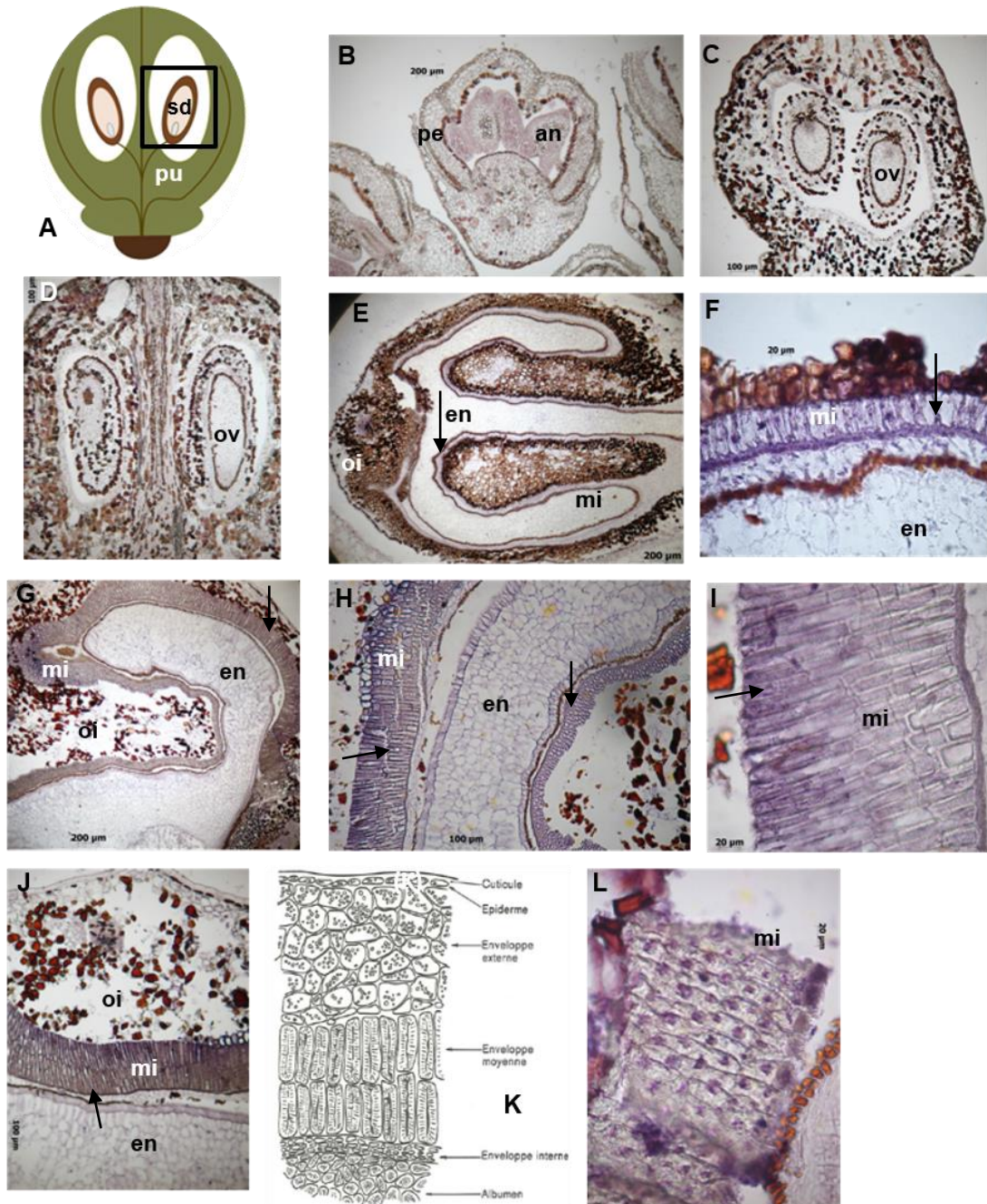
Fig. 7. *In situ* hybridization of *VvAGL11* in 'Chardonnay' reproductive tissues and organs, from flowers to seeds after 6 weeks of fruit development. **(A)** Schematic berry. No hybridization signal is visible in **(B)** H1 flower stage, **(C)** pre anthesis flower stage and **(D)** fruit set flower stage. *VvAGL11* expression is detectable in the medium integument of **(E-F)** 2 weeks seeds and **(G-J)** 4 weeks seeds (black arrows). No hybridization signal is visible in **(L)** 6 week seeds, compared to the control slides (Supplementary Fig. 2). **(K)** Squematic figure of the grapevine seed layers (Pratt, 1971). Anther (an), endosperm (en), medium integument (mi), outer integument (oi), petal (pe), pulp (pu), seed (sd).

57
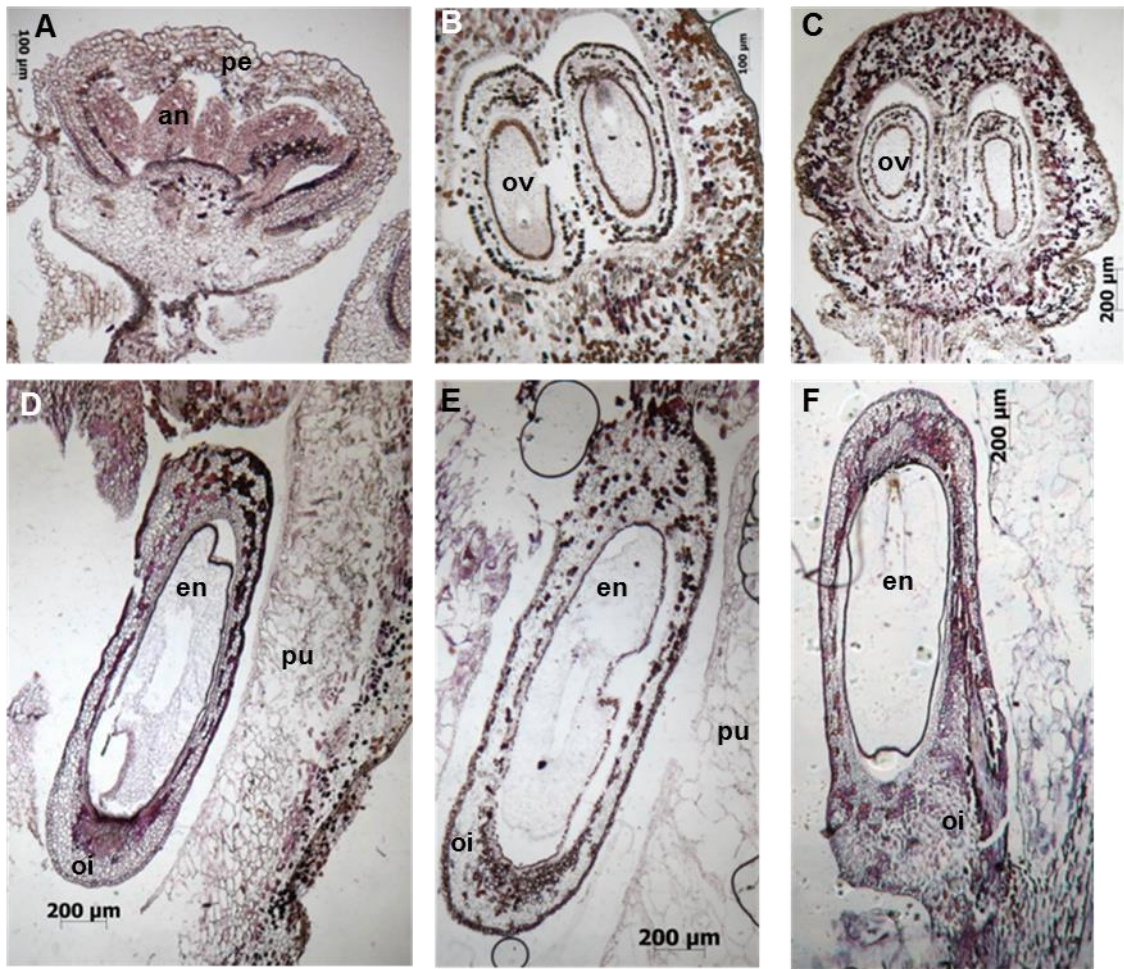
Fig. 8. *In situ* hybridization of *VvAGL11* in 'Sultanina' from flowers to 6 weeks of fruit development. No hybridization signal is visible in H1 flower stage **(A)**, pre anthesis flower stage **(B)**, fruit set flower stage **(C)** and in 2 **(D)**, 4 **(E)** and 6 **(F)** weeks of fruit development. All samples compared with control slides (Supplementary Fig. 3). Anther (an), endosperm (en), medium integument (mi), outer integument (oi), petal (pe), pulp (pu).
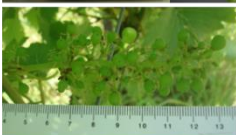
Supplementary Material

Supplementary Table 1. Primers designed for the amplification and sequencing of *VvAGL11*.

CDS+INTRON_AGL11_R_ 1	CAT CAG CAG ATG CCA GAT
CDS+INTRON_AGL11_F_ 1	CAA TAA ACC TAA CAG TGT AGT GAA C
CDS+INTRON_AGL11_R_ 2	GGA CAG TGG AAT ACA GAT TC
CDS+INTRON_AGL11_F_ 2	TAG GAA GGG ATT ACA TGG G
CDS+INTRON_AGL11_R_ 3	GAT GGC ATT GAG CAA ACA
CDS+INTRON_AGL11_F_ 3	CTA GGC AAC AAC CAG TTA G
CDS+INTRON_AGL11_R_ 4	ATA AGG AAG ACT TCT CCC C
CDS+INTRON_AGL11_F_ 4	CCC ATT ACA TCT TTG TGT GG
CDS+INTRON_AGL11_R_ 5	CTT TTT CTC CTA CTT TTC CTC
CDS+INTRON_AGL11_F_ 5	CGA TCT TAC ATG AGA TTT TAC C
CDS+INTRON_AGL11_R_ 6	TTG GAG CAT CGT CTT AGA G
CDS+INTRON_AGL11_F_ 6	GAGGAAAAGTAGGAGAAAAG
CDS+INTRON_AGL11_R_ 7	AAA CCC CAC TGT GAT AGG
CDS+INTRON_AGL11_F_ 7	GGA TGG TGT CAT AAT GAT AGG
CDS+INTRON_AGL11_R_ 8	GAA ATA TCA TAG TCA TCC TCA C
CDS+INTRON_AGL11_F_ 8	TAA GGT GTC GAA GCC ATG
CDS+INTRON_AGL11_R_ 9	TGA GTT TCT TTG TGT GGA C
CDS+INTRON_AGL11_F_ 9	GAG AGC CTA TTT GGG ATA AC
CDS+INTRON_AGL11_R_ 10	GAG TTT GTA TGG AGA ATA GCA G
CDS+INTRON_AGL11_F_ 10	GCA AAG CAG TTA TTG AAA GC
CDS+INTRON_AGL11_R_ 11	GGA TTT GGA TTG ATT CAT TAA C
CDS+INTRON_AGL11_F_ 11	AGG AGA AAC CAG GAC AAG
CDS+INTRON_AGL11_R_ 12	CAA ATG CTC AGC CAG ATT AG
CDS+INTRON_AGL11_F_ 12	TGC CCA AGT AAG AAA ACT TC
CDS+INTRON_AGL11_R_ 13	CGG TAT CAA CTG TTG TGC
CDS+INTRON_AGL11_F_ 13	GGT AAC GAC ACC TAG ACA C
CDS+INTRON_AGL11_R_ 14	GCT TGA AAT GTC TCA TTA GC
CDS+INTRON_AGL11_F_ 14	CGT ATA TCT CCG AAC CAA G
CDS+INTRON_AGL11_R_ 15	GGC GGA TAT TTT CTC CCA TC
CDS+INTRON_AGL11_F_ 15	CTC GGG TAC ACT CAT CTT TC
CDS+INTRON_AGL11_R_ 16	TGA TTA TCA ACA CCA AAC ATG GC
CDS+INTRON_AGL11_F_ 16	TTC AAG TTC GAC CTA ATT CCT GG
Promo_AGL11_R_ 1	GAT CTC GAT CTT TCC TCT CCC
Promo_AGL11_F_ 1	CCT CAC CCT ATT TTT CGA ACA AC
Promo_AGL11_R_ 2	GAG ATC TGA ATG ATG ATA GAC TGA G
Promo_AGL11_F_ 2	CTT GAG GGG AAA AAG CCA G
Promo_AGL11_R_ 3	CAT AAA CCC AGG ATA TGG ATG AG
Promo_AGL11_F_ 3	TTG CCT ACA TAT GAA CAT CTG C
Promo_AGL11_R_ 4	ATT TGG AGA GAT GGG CAC TG
Promo_AGL11_F_ 4	AAA GTC ACT TTC TAC ATC TGC AC
Promo_AGL11_R_ 5	GGA GAG ATG TGA GAC GGA TAT G

Promo_AGL11_F_5	AGA AGA AAG TTT GCA GTT TGT G
Promo_AGL11_R_6	CAA AGT CGG ATA CAA GAC ATA GTA G
Promo_AGL11_F_6	CCA CTG ATA TGG ATT GAT TTG CC

Supplementary Table 2. Stages of development of grapevine reproductive organs sampled for the evaluation of *VvAGL11* transcriptional profile (except H1) and for the *in situ* hybridization experiment. Dates of harvesting, the phenological stage according to Baggiolini (1952), grapevine cultivars and a representative picture of each sample are represented.

Dates	Phenological stage (Baggiolini, 1952)	Sample	Cultivar	
09/11/12	H1	Flowers at inicial development	Chardonnay	
08/30/12			Sultanina	
10/08/12	Pre anthesis	Flowers before anthesis	Chardonnay	
10/08/12			Sultanina	
10/22/12	Fruit-set	Flowers in anthesis	Chardonnay	
10/08/12			Sultanina	
11/05/12	2 weeks of development	Fruits (Pulp and seeds)	Chardonnay	
11/22/12			Sultanina	
11/19/12	4 weeks of development	Fruits (Pulp and seeds)	Chardonnay	
11/05/12			Sultanina	
12/03/12	6 weeks of development	Fruits (Pulp and seeds)	Chardonnay	
11/19/12			Sultanina	
01/02/13	Maturation	Fruits (Pulp and seeds)	Chardonnay	
12/17/12			Sultanina	

```
CLUSTAL multiple sequence alignment by MUSCLE (3.8)


su_mut                 CCACTGATATGGATTGATTTGCCTTTTTAATTGAAGACCCTTTTCTGTTGGTAACTTTGT
su_Di Genova           CCACTGATATGGATTGATTTGCCTTTTTAATTGAAGACCCTTTTCTGTTGGTAACTTTGT
ch2                    CCACTGATATGGATTGATTTGCCTTTTTAATTGAAGACCCTTTTCTGTTGGTAACTTTGT
VIT_218S0041G01880.2   CCACTGATATGGATTGATTTGCCTTTTTAATTGAAGACCCTTTTCTGTTGGTAACTTTGT
su_wt                  CCACTGATATGGATTGATTTGCCTTTTTAATTGAAGACCCTTTTCTGTTGGTAACTTTGT
ch1                    CCACTGATATGGATTGATTTGCCTTTTTAATTGAAGACCCTTTTCTGTTGGTAACTTTGT
                       ************************************************************


su_mut                 GAAGGCATGAACGAAGTTGATTACTGAAAATATTAGCAACATTACTAGGGTTTTGGCGGA
su_Di Genova           GAAGGCATGAACGAAGTTGATTACTGAARATATTAGCAACATTACTAGGGTTTTGGCGGA
ch2                    GAAGGCATGAACGAAGTTGATTACTGAAGATATTAGCAACATTACTAGGGTTTT---GGA
VIT_218S0041G01880.2   GAAGGCATGAACGAAGTTGATTACTGAAGATATTAGCAACATTACTAGGGTTTT---GGA
su_wt                  GAAGGCATGAACGAAGTTGATTACTGAAGATATTAGCAACATTACTAGGGTTTT---GGA
ch1                    GAAGGCATGAACGAAGTTGATTACTGAAGATATTAGCAACATTACTAGGGTTTT---GGA
                       ***************************** ***********************   ***


su_mut                 GATGAGACTGTAAAGTTGGCTAAAACCCTAGAGGCATGTTGTTTTTTAATAATTGGAGAT
su_Di Genova           GATGAGACTGTAAAGTTGGCTAAAACCCTAGAGGCATGTTGTTTTTTAATAATTGGAGAT
ch2                    GATGAGACTGTAAAGTTGGCTAAAACCCTAGAGGCATGTTGTTTTTTAATAATTGGAGAT
VIT_218S0041G01880.2   GATGAGACTGTAAAGTTGGCTAAAACCCTAGAGGCATGTTG-TTTTTAATAATTGGAGAT
su_wt                  GATGAGACTGTAAAGTTGGCTAAAACCCTAGAGGCATGTTG-TTTTTAATAATTGGAGAT
ch1                    GATGAGACTGTAAAGTTGGCTAAAACCCTAGAGGCATGTTG-TTTTTAATAATTGGAGAT
                       ***************************************** *****************


su_mut                 ACCATATATGTATGTTGCGAGTGCAGATATACGTGGAAAGCACAAAAATCCTAACCTGTT
su_Di Genova           ACCATATATGTATGTTGCGAGTGCAGATATACGTGGAAAGCACAAAAATCCTAACCTGTT
ch2                    ACCATATATGTATGTTGCGAGTGCAGATATACGTGGAAAGCACAAAAATCCTAACCTGTT
VIT_218S0041G01880.2   ACCATATATGTATGTTGCGAGTGCAGATATACGTGGAAAGCACAAAAATCCTAACCTGTT
su_wt                  ACCATATATGTATGTTGCGAGTGCAGATATACGTGGAAAGCACAAAAATCCTAACCTGTT
ch1                    ACCATATATGTATGTTGCGAGTGCAGATATACGTGGAAAGCACAAAAATCCTAACCTGTT
                       ************************************************************


su_mut                 AAACTGTAATTTGGGAAGATATTGTGTTTCTTTTACGTGTTGGATACAACATTGGGTTGT
su_Di Genova           AAACTGTAATTTGGGAAGATATTGTGTTTCTTTTACGTGTTGGATACAACATTGGGTTGT
ch2                    AAACTGTAATTTGGGAAGATATTGTGTTTCTTTTACGTGTTGGATACAACATTGGGTTGT
VIT_218S0041G01880.2   AAACTGTAATTTGGGAAGATATTGTGTTTCTTTTACGTGTTGGATACAACATTGGGTTGT
su_wt                  AAACTGTAATTTGGGAAGATATTGTGTTTCTTTTACGTGTTGGATACAACATTGGGTTGT
ch1                    AAACTGTAATTTGGGAAGATATTGTGTTTCTTTTACGTGTTGGATACAACATTGGGTTGT
                       ************************************************************


su_mut                 GGCCTTGAAGAAAATAAAGAAGAGAAGAAAGTTTGCAGTTTATGGTGGGAATTTGGAACT
su_Di Genova           GGCCTTGAAGAAAATAAAGAAGAGAAGAAAGTTTGCAGTTTRTGGTGGGAATTTGGAACT
ch2                    GGCCTTGAAGAAAATAAAGAAGAGAAGAAAGTTTGCAGTTTATGGTGGGAATTTGGAACT
VIT_218S0041G01880.2   GGCCTTGAAGAAAATAAAGAAGAGAAGAAAGTTTGCAGTTTGTGGTGGGAATTTGGAACT
su_wt                  GGCCTTGAAGAAAATAAAGAAGAGAAGAAAGTTTGCAGTTTGTGGTGGGAATTTGGAACT
ch1                    GGCCTTGAAGAAAATAAAGAAGAGAAGAAAGTTTGCAGTTTGTGGTGGGAATTTGGAACT
                       ***************************************** *****************


su_mut                 GCAAAGCTGTTTACATTATAAAACTACTATGTCTTGTATCCGACTTTGGGTAATAAGCCA
su_Di Genova           GCAAAGCTGTTTACATTATAAAACTACTATGTCTTGTATCCGACTTTGGGTAATAAGCCA
ch2                    GCAAAGCTGTTTACATTATAAAACTACTATGTCTTGTATCCGACTTTGGGTAATAAGCCA
VIT_218S0041G01880.2   GCAAAGCTGTTTACATTATAAAACTACTATGTCTTGTATCCGACTTTGGGTAATAAGCCA
su_wt                  GCAAAGCTGTTTACATTATAAAACTACTATGTCTTGTATCCGACTTTGGGTAATAAGCCA
ch1                    GCAAAGCTGTTTACATTATAAAACTACTATGTCTTGTATCCGACTTTGGGTAATAAGCCA
                       ************************************************************


su_mut                 TTCGGAAGTCTTTTTCTTCCTCAACCC------CTCTCTCTCTCTCTCTCTTTGAAGAAG
su_Di Genova           TTCGGAAGTCTTTTTCTTCCTCAACCC----CTCTCTCTCTCTCTCTCTCTTTGAAGAAG
ch2                    TTCGGAAGTCTTTTTCTTCCTCAACCCCTCTCTCTCTCTCTCTCTCTCTCTTTGAAGAAG
VIT_218S0041G01880.2   TTCGGAAGTCTTTTTCTTCCTCAACCC----CTCTCTCTCTCTCTCTCTCTTTGAAGAAG
su_wt                  TTCGGAAGTCTTTTTCTTCCTCAACCC----CTCTCTCTCTCTCTCTCTCTTTGAAGAAG
ch1                    TTCGGAAGTCTTTTTCTTCCTCAACCC----CTCTCTCTCTCTCTCTCTCTTTGAAGAAG
                       **************************           ************************
```

```
su_mut                  TCATTTTCTTCATCTGCACCGCCCATTCTCTCTCGCTATTGTCATCTTTCTCCTC--CTC
su_Di Genova            TCATTTTCTTCATCTGCACCGCCCATTCTCTCTCGCTATTGTCATCTTTCTCCTCCTCTC
ch2                     TCATTTTCTTCATCTGCACCGCCCATTCTCTCTCGCTATTGTCATCTTTCTCCTC-----
VIT_218S0041G01880.2    TCATTTTCTTCATCTGCACCGCCCATTCTCTCTCGCTATTGTCATCTTTCTCCTC--TTC
su_wt                   TCATTTTCTTCATCTGCACCGCCCATTCTCTCTCGCTATTGTCATCTTTCTCCTC--TTC
ch1                     TCATTTTCTTCATCTGCACCGCCCATTCTCTCTCGCTATTGTCATCTTTCTCCTC--TTC
                        *******************************************************

su_mut                  TCTCTCTCTCTCTCTCTCTCTCTCTCTATTGTACTATCTCTCTTCTCTCTCTATCTCTCT
su_Di Genova            TCTCTCTCTCTCTCTCTCTCTCTCTCTATTGTACTATCTCTCTTCTCTCTCTATCTCTCT
ch2                     -----TTCTCTCTCTCTCTCTCTCTCTATTGTACTATCTCTCTTCTCTCTCTATCTCTCA
VIT_218S0041G01880.2    TCTCTCTCTCTCTCTCTCTCTCTCTCTCTATTGTACTATCTCTCTTCTCTCTCTATCTCTCT
su_wt                   TCTCTCTCTCTCTCTCTCTCTCTCTCTATTGTACTATCTCTCTTCTCTCTCTATCTCTCT
ch1                     TCTCTCTCTCTCTCTCTCTCTCTCTCTATTGTACTATCTCTCTTCTCTCTCTATCTCTCT
                             ********************************************************

su_mut                  TTATTGCTCTCTCTCTCCCTTTCCCTCTCCCTCTCCCTCTCCCTCCCTCTCTCTCTCTCT
su_Di Genova            TTATTGCTCTCTCTCTCCCTTTCCCTCTCCCTCTCC--CTCCCTCTCTCTCTCTCTCTCT
ch2                     TTATTGCTCTCTCTCTCCCTTTCCCTCTCCCTCTCC--------CTCCCTCTCCCTCTCT
VIT_218S0041G01880.2    TTATTGCTCTCTCTCTCCCTTTCCCTCTCCCTCTCC--CTCCCTCTCTCTCTCTCTCTCT
su_wt                   TTATTGCTCTCTCTCTCCCTTTCCCTCTCCCTCTCC--CTCCCTCTCTCTCTCTCTCTCT
ch1                     TTATTGCTCTCTCTCTCCCTTTCCCTCTCCCTCTCC--CTCCCTCTCTCTCTCTCTCTCT
                        ***********************************         *  *  *****  ******

su_mut                  CAAAGTCACTTTCTACATCTGCACCACCCACTCTCTCTCTCTCTCTCTCTCTCTCTTTCA
su_Di Genova            CAAAGTCACTTTCTACATCTGCACCACCCACTCTCTCTCTCTCTCTC--------TCA
ch2                     CAAAGTCACTTTCTACATCTGCACCACCCACTCTCTCTCTCTCTCTC------------TCA
VIT_218S0041G01880.2    CAAAGTCACTTTCTACATCTGCACCACCCACTCTCTCTCTCTCTCTCTC--------TCA
su_wt                   CAAAGTCACTTTCTACATCTGCACCACCCACTCTCTCTCTCTCTCTCTC--------TCA
ch1                     CAAAGTCACTTTCTACATCTGCACCACCCACTCTCTCTCTCTCTCTCTC--------TCA
                        **********************************************          ***

su_mut                  TATCCGTCTCACATCTCTCCATTGTGGTATATATATATAAGGACTAAACACAAGTCTTAA
su_Di Genova            TATCCGTCTCACATCTCTCCATTGTGGTATATATATATAAGGACTAAACACAAGTCTTAW
ch2                     TATCCGTCTCACATCTCTCCATTGTGGTATATATATATAAGGACTAAACACAAGTCTTAA
VIT_218S0041G01880.2    TATCCGTCTCACATCTCTCCATTGTGGTATATATATATAAGGACTAAACACAAGTCTTAA
su_wt                   TATCCGTCTCACATCTCTCCATTGTGGTATATATATATAAGGACTAAACACAAGTCTTAA
ch1                     TATCCGTCTCACATCTCTCCATTGTGGTATATATATATAAGGACTAAACACAAGTCTTAA
                        ***********************************************************

su_mut                  GGCACTTTGGCTTCATTGGGATACGCGTTTTTGCACAGCCCGAAATTTTCCGAAAGCTGA
su_Di Genova            GGCACTTTGGCTTCATTGGGATACGCGTTTTTGCACAGCCCGAAATTTTCCGAAAGCTGA
ch2                     GGCACTTTGGCTTCATTGGGATACGCGTTTTTGCACAGCCCGAAATTTTCCGAAAGCTGA
VIT_218S0041G01880.2    GGCACTTTGGCTTCATTGGGATACGCGTTTTTGCACAGCCCGAAATTTTCCGAAAGCTGA
su_wt                   GGCACTTTGGCTTCATTGGGATACGCGTTTTTGCACAGCCCGAAATTTTCCGAAAGCTGA
ch1                     GGCACTTTGGCTTCATTGGGATACGCGTTTTTGCACAGCCCGAAATTTTCCGAAAGCTGA
                        ***********************************************************

su_mut                  AGTCGCCGGATTTTGGAAACAAGGTGTGTAGGTGATCTTTTAACAGAAACGTTTCACCAC
su_Di Genova            AGTCGCCGGATTTTGGAAACAAGGTGTGTAGGTGATCTTTTAACAGAAACGTTTCACCAC
ch2                     AGTCGCCGGATTTTGGAAACAAGGTGTGTAGGTGATCTTTTAACAGAAACGTTTCACCAC
VIT_218S0041G01880.2    AGTCGCCGGATTTTGGAAACAAGGTGTGTAGGTGATCTTTTAACAGAAACGTTTCACCAC
su_wt                   AGTCGCCGGATTTTGGAAACAAGGTGTGTAGGTGATCTTTTAACAGAAACGTTTCACCAC
ch1                     AGTCGCCGGATTTTGGAAACAAGGTGTGTAGGTGATCTTTTAACAGAAACGTTTCACCAC
                        ***********************************************************

su_mut                  CAAAATTTCCACAATCAACCATTCTCTCTCTGTGAAAACGTTTCGTGCATAACTGGGTAA
su_Di Genova            CAAAATTTCCACAATCAACCATTCTCTCTCTGTGAAAACGTTTCGTGCATAACTGGGTAA
ch2                     CAAAATTTCCACAATCAACCATTCTCTCTCTGTGAAAACGTTTCGTGCATAACTGGGTAA
VIT_218S0041G01880.2    CAAAATTTCCACAATCAACCATTCTCTCTCTGTGAAAACGTTTCGTGCATAACTGGGTAA
su_wt                   CAAAATTTCCACAATCAACCATTCTCTCTCTGTGAAAACGTTTCGTGCATAACTGGGTAA
ch1                     CAAAATTTCCACAATCAACCATTCTCTCTCTGTGAAAACGTTTCGTGCATAACTGGGTAA
                        ***********************************************************

su_mut                  TCTTAGATCTGCTCCCCTCCACACCACAGAATCTACTTTTGCCTACATATGAACATCTGC
su_Di Genova            TCTTAGATCTGCTCCCCTCCACACCACAGAATCTACTTTTGCCTACATATGAACATCTGC
ch2                     TCTTAGATCTGCTCCCCTCCACACCACAGAATCTACTTTTGCCTACATATGAACATCTGC
VIT_218S0041G01880.2    TCTTAGATCTGCTCCCCTCCACACCACAGAATCTACTTTTGCCTACATATGAACATCTGC
su_wt                   TCTTAGATCTGCTCCCCTCCACACCACAGAATCTACTTTTGCCTACATATGAACATCTGC
ch1                     TCTTAGATCTGCTCCCCTCCACACCACAGAATCTACTTTTGCCTACATATGAACATCTGC
```

```
                         ************************************************************

su_mut              TTTCCATTTCTTCCTCTTTCTTTTGTCAGTGCCCATCTCTCCAAATTTACTTCCACCTCT
su_Di Genova        TTTCCATTTCTTCCTCTTTCTTTTGTCAGTGCCCATCTCTCCAAATTTACTTCCACCTCT
ch2                 TTTCCATTTCTTCCTCTTTCTTTTGTCAGTGCCCATCTCTCCAAATTTACTTCCACCTCT
VIT_218S0041G01880.2 TTTCCATTTCTTCCTCTTTCTTTTGTCAGTGCCCATCTCTCCAAATTTACTTCCACCTCT
su_wt               TTTCCATTTCTTCCTCTTTCTTTTGTCAGTGCCCATCTCTCCAAATTTACTTCCACCTCT
ch1                 TTTCCATTTCTTCCTCTTTCTTTTGTCAGTGCCCATCTCTCCAAATTTACTTCCACCTCT
                    ************************************************************


su_mut              TACATTTCTTACCATTCTTTTTAGATTTCTTGGCTTGATTTACTCTCTTCTTCCTGCAA
su_Di Genova        TACATTTCTTACCATTCTTTTTAGATTTCTTGGCTTGATTTACTCTCTTCTTCCTGCAA
ch2                 TACATTTCTTACCATTCTTTTTAGATTTCTTGGCTTGATTTACTCTCTTCTTCCTGCAA
VIT_218S0041G01880.2 TACATTTCTTACCATTCTTTTTAGATTTCTTGGCTTGATTTACTCTCTTCTTCCTGCAA
su_wt               TACATTTCTTACCATTCTTTTTAGATTTCTTGGCTTGATTTACTCTCTTCTTCCTGCAA
ch1                 TACATTTCTTACCATTCTTTTTAGATTTCTTGGCTTGATTTACTCTCTTCTTCCTGCAA
                    ************************************************************


su_mut              CACTTTACTCTTCAGTTCTTGATTCTCTTTTGCCTTCTTCATGCATTTGTTTCCATT{CTT}
su_Di Genova        CACTTTACTCTTCAGTTCTTGATTCTCTTTTGCCTTCTTCATGCATTTGTTTCCATT{CTT}
ch2                 CACTTTACTCTTCAGTTCTTGATTCTCTTTTGCCTTCTTCATGCATTTGTTTCCATT{CTT}
VIT_218S0041G01880.2 CACTTTACTCTTCAGTTCTTGATTCTCTTTTGCCTTCTTCATGCATTTGTTTCCATT{CTT}
su_wt               CACTTTACTCTTCAGTTCTTGATTCTCTTTTGCCTTCTTCATGCATTTGTTTCCATT{CTT}
ch1                 CACTTTACTCTTCAGTTCTTGATTCTCTTTTGCCTTCTTCATGCATTTGTTTCCATT{CTT}
                    ************************************************************


su_mut              {TAATTAG}TCATTTTCTTATTCTAAACTTTCTTTTCCCTTTTTTCGTTATTTTCAGAATGC
su_Di Genova        {TAATTAG}TCATTTTCTTATTCTAAACTTTCTTTTCCCTTTTTTCGTTATTTTCAGAATGC
ch2                 {TAATTAG}TCATTTTCTTATTCTAAACTTTCTTTTCCCTTTTTTCGTTATTTTCAGAATGC
VIT_218S0041G01880.2 {TAATTAG}TCATTTTCTTATTCTAAACTTTCTTTTCCCTTTTTTCGTTATTTTCAGAATGC
su_wt               {TAATTAG}TCATTTTCTTATTCTAAACTTTCTTTTCCCTTTTTTCGTTATTTTCAGAATGC
ch1                 TAATTAGTCATTTTCTTATTCTAAACTTTCTTTTCCCTTTTTTCGTTATTTTCAGAATGC
                    ************************************************************


su_mut              AGTTTGATTATTTTGCCTTTTTTTTCATGTTAAAACAGTATTTGTTTTGGTAGTTCTCAG
su_Di Genova        AGTTTGATTATTTTTCC-TTTTTTTCATGTTAAAACAGTATTTGTTTTGGTAGTTCTCAG
ch2                 AGTTTGATTATTTTGCC-TTTTTTTCATGTTAAAACAGTATTTGTTTTGGTAGTTTTCAG
VIT_218S0041G01880.2 AGTTTGATTATTTTTCC-TTTTTTTCATGTTAAAACAGTATTTGTTTTGGTAGTTTTCAG
su_wt               AGTTTGATTATTTTTCC-TTTTTTTCATGTTAAAACAGTATTTGTTTTGGTAGTTTTCAG
ch1                 AGTTTGATTATTTTTCC-TTTTTTTCATGTTAAAACAGTATTTGTTTTGGTAGTTTTCAG
                    ************* ** *********************************** ****


su_mut              TTTATTTTTTCCATAAATTTCTGTTTCAAAAACCTCTTGAAGGGAAAAAGCCAGAATTTT
su_Di Genova        TTTATTTTTTCCATAAATTTCTGTTTCAAAAACCTCTTGARGGGAAAAAGCCAGAATTTT
ch2                 TTTATTTTTTCCATAAATTTCTGTTTCAAAAACCTCTTGAAGGGAAAAAGCCAGAATTTT
VIT_218S0041G01880.2 TTTATTTTTTCCATAAATTTCTGTTTCAAAAACCTCTTGAGGGGAAAAAGCCAGAATTTT
su_wt               TTTATTTTTTCCATAAATTTCTGTTTCAAAAACCTCTTGAGGGGAAAAAGCCAGAATTTT
ch1                 TTTATTTTTTCCATAAATTTCTGTTTCAAAAACCTCTTGAGGGGAAAAAGCCAGAATTTT
                    *************************************** ******************


su_mut              TTTTCCTGCGTTCTGGTCTTAAGCTTCTCATCCATATCCTGGGTTTATGGGAAATGTGTT
su_Di Genova        TTTTCCTGCGTTCTGGTCTTAAGCTTCTCATCCATATCCTGGGTTTATGGGAAATGTGTT
ch2                 TTTTCCTGCGTTCTGGTCTTAAGCTTCTTATCCATATCCTAGGTTTATGGGAAATGTGTT
VIT_218S0041G01880.2 TTTTCCTGCGTTCTGGTCTTAAGCTTCTCATCCATATCCTGGGTTTATGGGAAATGTGTT
su_wt               TTTTCCTGCGTTCTGGTCTTAAGCTTCTCATCCATATCCTGGGTTTATGGGAAATGTGTT
ch1                 TTTTCCTGCGTTCTGGTCTTAAGCTTCTCATCCATATCCTGGGTTTATGGGAAATGTGTT
                    **************************** ********** ******************


su_mut              AAGGGTCATGGGGTTTTTGAGAAATGTG----GAGAGAGAGAGAGAGAGAGAGAGAGAGA
su_Di Genova        AAGGGTTATGGGGTTTTTGAGAAATGTG----GAGAGAGAGAGAGAGAGAGAGAGAGAGA
ch2                 AAGGGTCATGGGGTTTTTGAGAAATGTGGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGA
VIT_218S0041G01880.2 AAGGGTTATGGGGTTTTTGAGAAATGTG--------------------GAGAGAGAGA
su_wt               AAGGGTTATGGGGTTTTTGAGAAATGTG--------------------GAGAGAGAGA
ch1                 AAGGGTTATGGGGTTTTTGAGAAATGTG--------------------GAGAGAGAGA
                    ****** ******************** *********


su_mut              GAGAGAGATGTGTAGACATGCACCCACTTGTAGTAGTATTAGTAGTGGAGATAGATCCTG
su_Di Genova        GAGAGAGATGTGTAGACATGCACCCACTTGTAGTAGTATTAGTAGTGGAGATAGATCCTG
ch2                 GAGAGAGATGTGTAGACATGCACCCACTTGTAGTAGTATTAGTAGTGGAGATAGATCCTG
```

```
VIT_218S0041G01880.2  GAGAGAGATGTGTAGACATGCACCCACTTGTAGTAGTATTAGTAGTGGAGATAGATCCTG
su_wt                 GAGAGAGATGTGTAGACATGCACCCACTTGTAGTAGTATTAGTAGTGGAGATAGATCCTG
ch1                   GAGAGAGATGTGTAGACATGCACCCACTTGTAGTAGTATTAGTAGTGGAGATAGATCCTG
                      ************************************************************


su_mut                GGTTGATTTTTCAAATGGTAGATCATGTTCTCTTCTCTTTGTTCTCATCTCTTCATGTTC
su_Di Genova          GGTTGATTTTTCAAATGGTAGATCATGTTCTCTTCTCTTTGTTCTCATCTCTTCATGTTC
ch2                   GGTTGATTTTTCAAATGGTAGATCATGTTCTCTTCTCTTTGTTCTCATCTCTTCATGTTC
VIT_218S0041G01880.2  GGTTGATTTTTCAAATGGTAGATCATGTTCTCTTCTCTTTGTTCTCATCTCTTCATGTTC
su_wt                 GGTTGATTTTTCAAATGGTAGATCATGTTCTCTTCTCTTTGTTCTCATCTCTTCATGTTC
ch1                   GGTTGATTTTTCAAATGGTAGATCATGTTCTCTTCTCTTTGTTCTCATCTCTTCATGTTC
                      ************************************************************


su_mut                TTTTTTCCTCAGTGGCTTACAGATTTTCTGTCTCCCAATCTTTTGGTTTCCATCTCTTTT
su_Di Genova          TTTTTTCCTCAGTGGCTTACAGATTTTCTGTCTCCCAATCTTTTGGTTTCCATCTCTTTT
ch2                   TTTTTTCCTCAGTGGCTTACAGATTTTCTGTCTCCCAATCTTTTGGTTTCCATCTCTTTT
VIT_218S0041G01880.2  TTTTTTCCTCAGTGGCTTACAGATTTTCTGTCTCCCAATCTTTTGGTTTCCATCTCTTTT
su_wt                 TTTTTTCCTCAGTGGCTTACAGATTTTCTGTCTCCCAATCTTTTGGTTTCCATCTCTTTT
ch1                   TTTTTTCCTCAGTGGCTTACAGATTTTCTGTCTCCCAATCTTTTGGTTTCCATCTCTTTT
                      ************************************************************


su_mut                CCAAGATCTCCTTATTTTCCTACATTATCATCATTATTTTATTATATACACAAGCCTCAC
su_Di Genova          CCAAGATCTCCTTATTTTCCTACATTATCATCATTATTTTATTATATACACAAGCCTCAC
ch2                   CCAAGGTCTCCTTATTTTCCTACATTATCATCATTATTTTATTATATACACAAGCCTCAC
VIT_218S0041G01880.2  CCAAGATCTCCTTATTTTCCTACATTATCATCATTATTTTATTATATACACAAGCCTCAC
su_wt                 CCAAGATCTCCTTATTTTCCTACATTATCATCATTATTTTATTATATACACAAGCCTCAC
ch1                   CCAAGATCTCCTTATTTTCCTACATTATCATCATTATTTTATTATATACACAAGCCTCAC
                      *****  *****************************************************


su_mut                CCTATTTTTCAAACAACATCTAACTTTCTCATTTCTCAGTCTATCATCATTCAGA-----
su_Di Genova          CCTATTTTTCGAACAACATCTAACTTTCTCATTTCTCAGTCTATCATCATTCAGATCTCT
ch2                   CCTATTTTTCAAACAACATCTAACTTTCTCATTTCTCAGTCTATCATCATTCAGATCTCT
VIT_218S0041G01880.2  CCTATTTTTCGAACAACATCTAACTTTCTCATTTCTCAGTCTATCATCATTCAGATCTCT
su_wt                 CCTATTTTTCGAACAACATCTAACTTTCTCATTTCTCAGTCTATCATCATTCAGATCTCT
ch1                   CCTATTTTTCGAACAACATCTAACTTTCTCATTTCTCAGTCTATCATCATTCAGATCTCT
                      ********** **********************************************


su_mut                --TCTCCTCTCTCCTCTTCACCCTCTACCAAAAACACACATTTTTTCGGTAGATCTTC
su_Di Genova          CCTCTCTCCTCTCTCCTCTTCACCCTCTACCAAAAACACACATTTTTTCGGTAGATCTTC
ch2                   CCTCTCTCCTCTCTCCTCTTCACCCTCTACCAAAAACACACATTTTTTCGGTAGATCTTC
VIT_218S0041G01880.2  CCTCTCTCCTCTCTCCTCTTCACCCTCTACCAAAAACACACATTTTTTCGGTAGATCTTC
su_wt                 CCTCTCTCCTCTCTCCTCTTCACCCTCTACCAAAAACACACATTTTTTCGGTAGATCTTC
ch1                   CCTCTCTCCTCTCTCCTCTTCACCCTCTACCAAAAACACACATTTTTTCGGTAGATCTTC
                       *************************************** ******************


su_mut                TCTCACAAATTACATGCATTATTACCCTTCATATGTCACTCATACTTCATTTT-----TT
su_Di Genova          TCTCACAAATTACATGCATTATTACCCTTCATATGTCACTCATACTTCAWTTT-----TT
ch2                   TCTCACAAATTACATGCATTATTACCCTTCATGTGTCACTCATACTTCAATTTTTTTATT
VIT_218S0041G01880.2  TCTCACAAATTACATGCATTATTACCCTTCATATGTCACTCATACTTCAATTTT----TT
su_wt                 TCTCACAAATTACATGCATTATTACCCTTCATATGTCACTCATACTTCAATTTT----TT
ch1                   TCTCACAAATTACATGCATTATTACCCTTCATATGTCACTCATACTTCAATTTT----TT
                      ******************************** ***************** ***     **


su_mut                TTTTTTTTTTAAAGCTTGAACAGATCTCCAACTCTTTCTCTAGTATTTTGTAATCTTTAG
su_Di Genova          TTTTTTTTTTTAAAGCTTGAACAGATCTCCAACTCTTTCTCTAGTATTTTGTAATCTTTAG
ch2                   TTTTATTTTTAAAGCTTGATCAGATCTCCAACTCTTTCTCTAGTATTTTGTAATCTTTAG
VIT_218S0041G01880.2  TTTTTTTTTTAAAGCTTGAACAGATCTCCAACTCTTTCTCTAGTATTTTGTAATCTTTAG
su_wt                 TTTTTTTTTTAAAGCTTGAACAGATCTCCAACTCTTTCTCTAGTATTTTGTAATCTTTAG
ch1                   TTTTTTTTTTAAAGCTTGAACAGATCTCCAACTCTTTCTCTAGTATTTTGTAATCTTTAG
                      ****  ************** ***************************************


su_mut                CAGTTTCATAAAGAGATGCTTTGGTAATTTTTTCTAATATTTTCAGTTGGTGATATAATA
su_Di Genova          CAGTTTCATAAAGAGATGCTTTGGTAAWTTTTTCTAATATTTTCAGTTGGTGATATAATA
ch2                   CAGTTTCATAAAGAGATGCTTTGGTAATTTTTTCTAATATTTTCAGTTGGTGATATAATA
VIT_218S0041G01880.2  CAGTTTCATAAAGAGATGCTTTGGTAAATTTTTCTAATATTTTCAGTTGGTGATATAATA
su_wt                 CAGTTTCATAAAGAGATGCTTTGGTAAATTTTTCTAATATTTTCAGTTGGTGATATAATA
ch1                   CAGTTTCATAAAGAGATGCTTTGGTAAATTTTTCTAATATTTTCAGTTGGTGATATAATA
                      **************************  *******************************
```

```
su_mut                  AAACAATGTATAGTACTACAAAAATTTTGTTCTTATATACCTAAGCCATTTTTTCTTTTG
su_Di Genova            WAACAATGTATAGTACTACAAAAATTTTGTTCTTATATACCTAAGCCATTTTTTCTTTTG
ch2                     TAACAATGTATAGTAGTACAAAAATTTTGTTCTTATATACCTAAGCCATTTTTTCTTTTG
VIT_218S0041G01880.2    TAACAATGTATAGTACTACAAAAATTTTGTTCTTATATACCTAAGCCATTTTTTCTTTTG
su_wt                   TAACAATGTATAGTACTACAAAAATTTTGTTCTTATATACCTAAGCCATTTTTTCTTTTG
ch1                     TAACAATGTATAGTACTACAAAAATTTTGTTCTTATATACCTAAGCCATTTTTTCTTTTG
                        ************** *********************************************


su_mut                  GTGCAATAAACCTAACAGTGTAGTGAACATGGGGAGAGGAAAGATCGAGATCAAGAGGAT
su_Di Genova            GTGCAATAAACCTAACAGTGTAGTGAACATGGGGAGAGGAAAGATCGAGATCAAGAGGAT
ch2                     GTGCAATAAACCTAACAGTGTAGTGAACATGGGGAGAGGAAAGATCGAGATCAAGAGGAT
VIT_218S0041G01880.2    GTGCAATAAACCTAACAGTGTAGTGAACATGGGGAGAGGAAAGATCGAGATCAAGAGGAT
su_wt                   GTGCAATAAACCTAACAGTGTAGTGAACATGGGGAGAGGAAAGATCGAGATCAAGAGGAT
ch1                     GTGCAATAAACCTAACAGTGTAGTGAACATGGGGAGAGGAAAGATCGAGATCAAGAGGAT
                        ************************************************************


su_mut                  CGAAAACACGACCAACCGTCAGGTCACATTCTGCAAGCGAAGGAATGGGCTTTTGAAGAA
su_Di Genova            CGAAAACACGACCAACCGTCAGGTCACATTCTGCAAGCGAAGGAATGGGCTTTTGAAGAA
ch2                     CGAAAACACGACCAACCGTCAGGTCACATTCTGCAAGCGAAGGAATGGGCTTTTGAAGAA
VIT_218S0041G01880.2    CGAAAACACGACCAACCGTCAGGTCACATTCTGCAAGCGAAGGAATGGGCTTTTGAAGAA
su_wt                   CGAAAACACGACCAACCGTCAGGTCACATTCTGCAAGCGAAGGAATGGGCTTTTGAAGAA
ch1                     CGAAAACACGACCAACCGTCAGGTCACATTCTGCAAGCGAAGGAATGGGCTTTTGAAGAA
                        ************************************************************


su_mut                  GGCTTATGAATTATCAGTGCTATGTGATGCAGAAGTTGCCCTCATCGTCTTCTCCAGCCG
su_Di Genova            GGCTTATGAATTATCAGTGCTATGTGATGCAGAAGTTGCCCTCATCGTCTTCTCCAGCCG
ch2                     GGCTTATGAATTATCAGTGCTATGTGATGCAGAAGTTGCCCTCATCGTCTTCTCCAGCCG
VIT_218S0041G01880.2    GGCTTATGAATTATCAGTGCTATGTGATGCAGAAGTTGCCCTCATCGTCTTCTCCAGCCG
su_wt                   GGCTTATGAATTATCAGTGCTATGTGATGCAGAAGTTGCCCTCATCGTCTTCTCCAGCCG
ch1                     GGCTTATGAATTATCAGTGCTATGTGATGCAGAAGTTGCCCTCATCGTCTTCTCCAGCCG
                        ************************************************************


su_mut                  CGGTCGAGTCTATGAGTACTCAAACAACAAGTAATAATTTTCTCCACCATTTCTTCAACC
su_Di Genova            CGGTCGAGTCTATGAGTACTCAAACAACAAGTAATAATTTTCTCCACCATTTCTTCAACC
ch2                     CGGTCGAGTCTATGAGTACTCAAACAACAAGTAATAATTTTCTCCACCATTTCTTCAACC
VIT_218S0041G01880.2    CGGTCGAGTCTATGAGTACTCAAACAACAAGTAATAATTTTCTCCACCATTTCTTCAACC
su_wt                   CGGTCGAGTCTATGAGTACTCAAACAACAAGTAATAATTTTCTCCACCATTTCTTCAACC
ch1                     CGGTCGAGTCTATGAGTACTCAAACAACAAGTAATAATTTTCTCCACCATTTCTTCAACC
                        ************************************************************


su_mut                  ATCTGCTGAATTTTCTATGTTCATCTTTCTCCATTTTGGTGCATCATACGGGCAATTAAT
su_Di Genova            ATCTGCTGAATTTTCTATGTTCATCTTTCTCCATTTTGGTGCATCATACGGGCAATTAAT
ch2                     ATCTGCTGAATTTTCTATGTTCATCTTTCTCCATTTTGGTGCATCATACTGGCAATTAAT
VIT_218S0041G01880.2    ATCTGCTGAATTTTCTATGTTCATCTTTCTCCATTTTGGTGCATCATACGGGCAATTAAT
su_wt                   ATCTGCTGAATTTTCTATGTTCATCTTTCTCCATTTTGGTGCATCATACGGGCAATTAAT
ch1                     ATCTGCTGAATTTTCTATGTTCATCTTTCTCCATTTTGGTGCATCATACGGGCAATTAAT
                        ************************************************* **********


su_mut                  GGTTTTATATTGATGAGATTAATATCATCAGAAGTTGCAGAACCCTAATGTTATTAAAGC
su_Di Genova            GGTTTTATATTGATGAGATTAATATCATCAGAAGTTGCAGAACCCTAATGTTATTAAAGC
ch2                     GGTTTTATATTGATGAGATTAATATCATTAGAAGTTGCAGAACCCTAATGTTGTTAAAGC
VIT_218S0041G01880.2    GGTTTTATATTGATGAGATTAATATCATCAGAAGTTGCAGAACCCTAATGTTATTAAAGC
su_wt                   GGTTTTATATTGATGAGATTAATATCATCAGAAGTTGCAGAACCCTAATGTTATTAAAGC
ch1                     GGTTTTATATTGATGAGATTAATATCATCAGAAGTTGCAGAACCCTAATGTTATTAAAGC
                        *************************** ********************** *******


su_mut                  AAAG-AAAAAAAAAAAGAACAGTGGAAGGAGGAGATGCAATAAATTTGAAAATCTAACTC
su_Di Genova            AAAG-AAAAAAAAAAAGAACAGTGGAAGGAGGAGATGCAATAAATTTGAAAATCTAACTC
ch2                     AAAGAAAAAAAAAAAAGAACAGTGGAAGGAGGAGATGCAATAAATTTGAAAATCTAACTC
VIT_218S0041G01880.2    AAAG-AAAAAAAAAAAGAACAGTGGAAGGAGGAGATGCAATAAATTTGAAAATCTAACTC
su_wt                   AAAG-AAAAAAAAAAAGAACAGTGGAAGGAGGAGATGCAATAAATTTGAAAATCTAACTC
ch1                     AAAG-AAAAAAAAAAAGAACAGTGGAAGGAGGAGATGCAATAAATTTGAAAATCTAACTC
                        ****  ******************************************************


su_mut                  ACTGCAGATTATGATTTTTTACGAGGGGAGTCAGATTCCTTTTGCTTTATTTAGGAAGGG
su_Di Genova            ACTGCAGATTATGATTTTTTAYGAGGGGAGTCAGATTCCTTTTGCTTTATTTAGGAAGGG
ch2                     ACTGCAGATTATGATTTTTTATGAGGGGAGTCAGATTCCTTTTGCTTTATTTAGGAAGGG
VIT_218S0041G01880.2    ACTGCAGATTATGATTTTTTATGAGGGGAGTCAGATTCCTTTTGCTTTATTTAGGAAGGG
su_wt                   ACTGCAGATTATGATTTTTTATGAGGGGAGTCAGATTCCTTTTGCTTTATTTAGGAAGGG
ch1                     ACTGCAGATTATGATTTTTTATGAGGGGAGTCAGATTCCTTTTGCTTTATTTAGGAAGGG
```

```
                        ******************** **************************************

su_mut                  ATTACATGGGTACCTGCTTTGATATTTATGAT-ATTTTCTTTCATCAAAATTTAATTAAT
su_Di Genova            ATTACATGGGTACCTGCTTTGATATTTATGAT-ATTTTCTTTCATCAAAATTTAATTAAT
ch2                     ATTACATGGGTACCTGCTTTGATATTTATGAT-ATTTTCTTTCATCAAAATTTAATTAAT
VIT_218S0041G01880.2    ATTACATGGGTACCTGCTTTGATATTTATGATAATTTTCTTTTATCAAAATTTA---AAT
su_wt                   ATTACATGGGTACCTGCTTTGATATTTATGATAATTTTCTTTTATCAAAATTTA---AAT
ch1                     ATTACATGGGTACCTGCTTTGATATTTATGATAATTTTCTTTTATCAAAATTTA---AAT
                        ******************************** ********* ***********   ***


su_mut                  GAAGGAAAATGCAGATAATATTCTGACATTTCATCAGGCATCTGCTGATGAATCTGAGAA
su_Di Genova            GAAGGAAAATGCAGATAATATTCTGACATTTCATCTGGCATCTGCTGATGAATCTGAGAA
ch2                     GAAGGAAAATGCAGATAATATTCTGACATTTCATCAGGCATCTGCTGATGAATCTGAGAA
VIT_218S0041G01880.2    GAAGGAAAATGCAGATAATATTCTGACATTTCATCTGGCATCTGCTGATGAATCTGAGAA
su_wt                   GAAGGAAAATGCAGATAATATTCTGACATTTCATCTGGCATCTGCTGATGAATCTGAGAA
ch1                     GAAGGAAAATGCAGATAATATTCTGACATTTCATCTGGCATCTGCTGATGAATCTGAGAA
                        ********************************** **********************


su_mut                  AACAATGTCTTCTTCAAGATTTTTCACTAATTACATCTTTGACTGATATAAGGAAAATAT
su_Di Genova            AACAATGTCTTCTTCAAGATTTTTCACTAATTACATCTTTGACTGATATAAGGAAAATAT
ch2                     AACAATGTCTTCTTCATGATTTTTCACTAATTGCATCTTTGACTGATATAAGGAAAATAT
VIT_218S0041G01880.2    AACAATTCTTCTTCATGATTTTTCACTAATTACATCTTTGACTGATATAAGGAAAATAT
su_wt                   AACAATTCTTCTTCATGATTTTTCACTAATTACATCTTTGACTGATATAAGGAAAATAT
ch1                     AACAATTCTTCTTCATGATTTTTCACTAATTACATCTTTGACTGATATAAGGAAAATAT
                        ****** ********* ************** **************************


su_mut                  GAGAAAGAACACTATACCTATAGTTAGAAACTTCTTCAAAGATTAACTATCAGAGGGTT[C]
su_Di Genova            SAGAAAGAACACTATACCTATAGTTAGAAACTTCTTCAAAGATTAACTATCAGAGGGTT[C]
ch2                     CAGAAAGAACACTTTACCTATAGTTAGAAACTTCTTCAAAGATTAACTATCAGAGGGTT[C]
VIT_218S0041G01880.2    CAGAAAGAACACTATACCTATAGTTAGAAACTTCTTCAAAGATTAACTATCAGAGGGTT[C]
su_wt                   CAGAAAGAACACTATACCTATAGTTAGAAACTTCTTCAAAGATTAACTATCAGAGGGTT[C]
ch1                     CAGAAAGAACACTATACCTATAGTTAGAAACTTCTTCAAAGATTAACTATCAGAGGGTT[C]
                        *********** *********************************************


su_mut                  [TTTTTATTG]TATGTTTAGTGCATTAAAA-----AAAAATTATAATGAAAAAATAAAAATA
su_Di Genova            [TTTTTATTG]TATGTTTAGTGCATTAAAAAAAAATAAAAATTATAATGAAAAAATAAAAATA
ch2                     [TTTTTATTG]TATGTTTAGTGCATTAAAAAAAATAAAAATTATAATGAAAAAATAAAAATA
VIT_218S0041G01880.2    [TTTTTATTG]TATGTTTAGTGCATTAAAAAAAATAAAAATTATAATGAAAAAATAAAAATA
su_wt                   [TTTTTATTG]TATGTTTAGTGCATTAAAAAAAATAAAAATTATAATGAAAAAATAAAAATA
ch1                     [TTTTTATTG]TATGTTTAGTGCATTAAAAAAAATAAAAATTATAATGAAAAAATAAAAATA
                        ************************        *************************


su_mut                  AAATA-GCAAAAAAACCGAAAAAGTTGAAATCCAACTATCAGTTACACTGGTAGTACTGA
su_Di Genova            AAATAGGAAAAAAAATCGAAAAAGTTGAAATCCAACTATCAGTTACACTGGTAGTGCTGA
ch2                     AAATAGGAAAAAAAACCGAAAAAGTTGAAATCCAACTATCAGTTACACTGGTAGTACTGA
VIT_218S0041G01880.2    AAATAGGAAAAAAAATCGAAAAAGTTGAAATCCAACTATCAGTTACACTGGTAGTGCTGA
su_wt                   AAATAGGAAAAAAAATCGAAAAAGTTGAAATCCAACTATCAGTTACACTGGTAGTGCTGA
ch1                     AAATAGGAAAAAAAATCGAAAAAGTTGAAATCCAACTATCAGTTACACTGGTAGTGCTGA
                        ***** * ******* ****************************************** ****


su_mut                  TATGCATGCAAGGGAAGACAATCTAGGCAACAGCCAGTTAGGGTTTCTTGTCCTAGCTAG
su_Di Genova            TATGCATGCAAGGGAAGACAATCTAGGCAACARCCAGTTAGGGTTTCTTGTCCTAGCTAG
ch2                     TATGCATGCAAGGGAAGACAATCTAGGCAACAGCCAGTTAGGGTTTCTTGTCCTAGCTAG
VIT_218S0041G01880.2    TATGCATGCAAGGGAAGACAATCTAGGCAACAACCAGTTAGGGTTTCTTGTCCTAGCTAG
su_wt                   TATGCATGCAAGGGAAGACAATCTAGGCAACAACCAGTTAGGGTTTCTTGTCCTAGCTAG
ch1                     TATGCATGCAAGGGAAGACAATCTAGGCAACAACCAGTTAGGGTTTCTTGTCCTAGCTAG
                        ******************************* *************************


su_mut                  TTCTTCCCATCTCTCCTTACTGTTCCTTTCCAATAATATTTAACATTGTAATCTGTGAGA
su_Di Genova            TTCTTCCCATCTCTCCTTACTGTTCCTTTCCAATAATATTTAACATTGTAATCTGTGAGA
ch2                     TTCTTCCCATCTCTCCTTACTGTTCCTTTCCAATAATATTTAACATTGTAATCTGTGAGA
VIT_218S0041G01880.2    TTCTTCCCATCTCTCCTTACTGTTCCTTTCCAATAATATTTAACATTGTAATCTGTGAGA
su_wt                   TTCTTCCCATCTCTCCTTACTGTTCCTTTCCAATAATATTTAACATTGTAATCTGTGAGA
ch1                     TTCTTCCCATCTCTCCTTACTGTTCCTTTCCAATAATATTTAACATTGTAATCTGTGAGA
                        ************************************************************


su_mut                  ATCTGTATTCCACTGTCCACAGTACAGTATTTAAGAAAAGGGTTATGGCCCTTTTCCTCT
su_Di Genova            ATCTGTATTCCACTGTCCACAGTACAGTATTTAAGAAAAGGGTTATGGCCCTTTTCCTCT
ch2                     ATCTGTATTCCACTGTCCACAGTACAGTATTTAAGAAAAGGGTTATGGCCCTTTTCCTCT
```

```
VIT_218S0041G01880.2 ATCTGTATTCCACTGTCCACAGTACAGTATTTAAGAAAAGGGTTATGGCCCTTTTCCTCT
su_wt                ATCTGTATTCCACTGTCCACAGTACAGTATTTAAGAAAAGGGTTATGGCCCTTTTCCTCT
ch1                  ATCTGTATTCCACTGTCCACAGTACAGTATTTAAGAAAAGGGTTATGGCCCTTTTCCTCT
                     ************************************************************


su_mut               TCCACTCTTTGTTCAAGTCTCAGTTTCTCTGCTCTTCTCTTTCCATTTCCAGCTTTGGGA
su_Di Genova         TCCACTCTTTGTTCAAGTCTCAGTTTCTCTGCTCTTCTCTTTCCATTTCCAGCTTTGGGA
ch2                  TCCACTCTTTGTTCAAGTCTCAGTTTCTCTGCTCTTCTCTTTCCATTTCCAGCTTTGGGA
VIT_218S0041G01880.2 TCCACTCTTTGTTCAAGTCTCAGTTTCTCTGCTCTTCTCTTTCCATTTCCAGCTTTGGGA
su_wt                TCCACTCTTTGTTCAAGTCTCAGTTTCTCTGCTCTTCTCTTTCCATTTCCAGCTTTGGGA
ch1                  TCCACTCTTTGTTCAAGTCTCAGTTTCTCTGCTCTTCTCTTTCCATTTCCAGCTTTGGGA
                     ************************************************************


su_mut               GGCTTGGAGCCCATGTATCATAAATCCTTCCCTTGTTTTTCTCCATCTTTTTGTTTTGGG
su_Di Genova         GGCTTGGAGCCCATGTATCATAAATCCTTCCCTTGTTTTTCTCCATCTTTTTGTTTTGGG
ch2                  GGCTTGGAGCCCATGTATCATAAATCCTTCCCTTGTTTTTCTCCATCTTTTTGTTTTGGG
VIT_218S0041G01880.2 GGCTTGGAGCCCATGTATCATAAATCCTTCCCTTGTTTTTCTCCATCTTTTTGTTTTGGG
su_wt                GGCTTGGAGCCCATGTATCATAAATCCTTCCCTTGTTTTTCTCCATCTTTTTGTTTTGGG
ch1                  GGCTTGGAGCCCATGTATCATAAATCCTTCCCTTGTTTTTCTCCATCTTTTTGTTTTGGG
                     ************************************************************


su_mut               TTTCTTCAGCCTAAAAGCTGTGAACCTTTCAATGGAGTCTTTTGGTCCTCTCTTCTCTTT
su_Di Genova         TTTCTTCAGCCTAAAAGCTGTGAACCTTTCAATGGAGTCTTTTGGTCCTCTCTTCTCTTT
ch2                  TTTCTTCAGCCTAAAAGCTGTGAACCTTTCAATGGAGTCTTTTGGTCCTCTCTTCTCTTT
VIT_218S0041G01880.2 TTTCTTCAGCCTAAAAGCTGTGAACCTTTCAATGGAGTCTTTTGGTCCTCTCTTCTCTTT
su_wt                TTTCTTCAGCCTAAAAGCTGTGAACCTTTCAATGGAGTCTTTTGGTCCTCTCTTCTCTTT
ch1                  TTTCTTCAGCCTAAAAGCTGTGAACCTTTCAATGGAGTCTTTTGGTCCTCTCTTCTCTTT
                     ************************************************************


su_mut               CCCATTCTCATTTAAAGTGCAGTAAAGTGATCACCTTTCACTGCTCTTTGTTTTTTTCAC
su_Di Genova         CCCATTCTCATTTAAAGTGCAGTAAAGTGATCASCTTTCACTGCTCTTTGTTTTTTTCAC
ch2                  CCCATTCTCATTTAAAGTGCAGTAAAGTGATCAGCTTTCACTGCTCTTTGTTTTTTTCAC
VIT_218S0041G01880.2 CCCATTCTCATTTAAAGTGCAGTAAAGTGATCAGCTTTCACTGCTCTTTGTTTTTTTCAC
su_wt                CCCATTCTCATTTAAAGTGCAGTAAAGTGATCAGCTTTCACTGCTCTTTGTTTTTTTCAC
ch1                  CCCATTCTCATTTAAAGTGCAGTAAAGTGATCAGCTTTCACTGCTCTTTGTTTTTTTCAC
                     ******************************** *************************


su_mut               TTGGTGTGGGCAATTTGTGGATATCAAA--TCAGAAATGGGACGGTGTACATCAGCACCC
su_Di Genova         TTGGTGTGGGCAATTTGTGGATATCAAA--TCAGAAATGGGACGGTGTACATCAGCACCC
ch2                  TTGGTGTGGGCAATTTGTGGATATCAAA--TCAGAAATGGGACGGTGTACATCAGCACCC
VIT_218S0041G01880.2 TTGGTGTGGGCAATTTGTGGATATCAAATCTCAGAAATGGGACGGTGTACATCAGCACCC
su_wt                TTGGTGTGGGCAATTTGTGGATATCAAATCTCAGAAATGGGACGGTGTACATCAGCACCC
ch1                  TTGGTGTGGGCAATTTGTGGATATCAAATCTCAGAAATGGGACGGTGTACATCAGCACCC
                     ***************************   *****************************


su_mut               ATTACATCTTTGTGTGGGTTTGAATAAATATTTCATATCAATCATTTCATGTTTGCTCAA
su_Di Genova         ATTACATCTTTGTGTGGGTTTGAATAAATATTTCATATCAATCATTTCATGTTTGMTCAA
ch2                  ATTACATCTTTGTGTGGGTTTGAATAAATATTTCATATCAATCATTTCATGTTTGCTCAA
VIT_218S0041G01880.2 ATTACATCTTTGTGTGGGTTTGAATAAATATTTCATATCAATCATTTCATGTTTGCTCAA
su_wt                ATTACATCTTTGTGTGGGTTTGAATAAATATTTCATATCAATCATTTCATGTTTGCTCAA
ch1                  ATTACATCTTTGTGTGGGTTTGAATAAATATTTCATATCAATCATTTCATGTTTGCTCAA
                     ******************************************************* ****


su_mut               TGCCATCCAAGATTTTTTTTCTTCACTTCATGTCTCATTTTCCTCCCCGATTTCTCCCTT
su_Di Genova         TGCCATCCAAGATTTTTTTTCTTCACTTCATGTCTCATTTTCCTCCCCGATTTCTCCCTT
ch2                  TGCCATCCAAGATTTTTTTTCTTCACTTCATGTCTCATTTTCCTCCTTGATTTCTCCCTT
VIT_218S0041G01880.2 TGCCATCCAAGATTTTTTTTCTTCACTTCATGTCTCATTTTCCTCCTTGATTTCTCCCTT
su_wt                TGCCATCCAAGATTTTTTTTCTTCACTTCATGTCTCATTTTCCTCCTTGATTTCTCCCTT
ch1                  TGCCATCCAAGATTTTTTTTCTTCACTTCATGTCTCATTTTCCTCCTTGATTTCTCCCTT
                     **********************************************  ************


su_mut               GTTCAAGGAAAATTGAAGATCTTCTCATAGTTGAGATAGTATTTAAAATAGGACATTGAT
su_Di Genova         GTTCAAGGAAAATTGAAGATCTTCTCATAGTTGAGATAGTATTTAAAATAGGACATTGAT
ch2                  GTTCAAGGAAAATTGAAGATCTTCTCATAGTTGAGATAGTATTTAAAATAGGACATTGAT
VIT_218S0041G01880.2 GTTCAAGGAAAATTGAAAATCTTCTCATAGTTGAGATAGTATTTAAAATAGGACATCGAT
su_wt                GTTCAAGGAAAATTGAAAATCTTCTCATAGTTGAGATAGTATTTAAAATAGGACATTGAT
ch1                  GTTCAAGGAAAATTGAAAATCTTCTCATAGTTGAGATAGTATTTAAAATAGGACATTGAT
                     ***************** ************************************** ***
```

```
su_mut                  CTTACATGAGATTTTACTTTTTTGTTTTCAAAATTTTAAATTATTTTTTATACTTTTTAA
su_Di Genova            CTTACATGAGATTTTACTTTTTTGTTTTCAAAATTTTAAATTATTTTTTATACTTTTTAA
ch2                     CTTACATGAGATTTTACCTTTTTTTTTTTCAAAATTTTAAATTATTTTTTATACTTTTTAA
VIT_218S0041G01880.2    CTTACATGAGATTTTACCTTTTTTTTTTTCAAAATTTTAAATTATTTTTTATACTTTTTAA
su_wt                   CTTACATGAGATTTTACCTTTTTTTTTTTCAAAATTTTAAATTATTTTTTATACTTTTTAA
ch1                     CTTACATGAGATTTTACCTTTTTTTTTTTCAAAATTTTAAATTATTTTTTATACTTTTTAA
                        ***************** ***** ***********************************


su_mut                  AAATACTTGTTTTTACCCTTAATCAATTAACAAACAAAAAAATAAAATGAAAAAAAAGAC
su_Di Genova            AAATACTTGTTTTTACCCTTAATCAATTAACAAACAAAAAAATAAAATGAAAAAAAAGAC
ch2                     AAATACTTGTTTTTACCCTTAATCAATTAACAAAAAAAATGA-AAAATG-AAAAAAAGAC
VIT_218S0041G01880.2    AAATACTTGTTTTTACCCTTAATCAATTAACAAAAAAAATGA-AAAATG-AAAAAAAGAC
su_wt                   AAATACTTGTTTTTACCCTTAATCAATTAACAAAAAAAATGA-AAAATG-AAAAAAAGAC
ch1                     AAATACTTGTTTTTACCCTTAATCAATTAACAAAAAAAATGA-AAAATG-AAAAAAAGAC
                        ******************************** ****   * ****** **********


su_mut                  CAATTTGAATTTTTTTTTTAATGTTGAGGGTGCA------------TGGGGGTGGGGGGG
su_Di Genova            CAATTTGAATTTTTTTTTTAATGTTGAGGGTGCA------------TGGGGGTGGGGGGG
ch2                     CAATTTGAATTTTTTTTTTAATGTTGAGGGTGCA------TGGGGGTGGGGGGTGGAGGGG
VIT_218S0041G01880.2    CAATTTGAATTTTTTTTTTAATGTTGAGGGTGCATGGGGGTGGGGGTGGGGGTGGAGGGG
su_wt                   CAATTTGAATTTTTTTTTTAATGTTGAGGGTGCA------TGGGGGTGGGGGGTGGAGGGG
ch1                     CAATTTGAATTTTTTTTTTAATGTTGAGGGTGCA------TGGGGGTGGGGGGTGGAGGGG
                        *********************************          ******** ****


su_mut                  AGAAGTCTTCCTTATTATTTATTAATTTTAATGCATTATTTTTAGTCAAATCCAATTTTA
su_Di Genova            AGAAGTCTTCCTTATTATTTATTAATTTTAATGCATTATTTTTAGTCAAATCCAATTWTA
ch2                     AGAAGTCTTCCTTATTATTTATTAATTTTAATGCATTATTTTTAGTCAAATCCAATTATA
VIT_218S0041G01880.2    AGAAGTCTTCCTTATTATTTATTAATTTTAATGCATTATTTTTAGTCAAATCCAATTATA
su_wt                   AGAAGTCTTCCTTATTATTTATTAATTTTAATGCATTATTTTTAGTCAAATCCAATTATA
ch1                     AGAAGTCTTCCTTATTATTTATTAATTTTAATGCATTATTTTTAGTCAAATCCAATTATA
                        ******************************************************** **


su_mut                  TTGCATAAAATTAATTAACATCATCTTTGATCATCTTTAAATCATATGGATTAAATCGTA
su_Di Genova            TTGCATAAAATTAATTAACATCATCTTTGATCATCTTTAAATCATATGGATTAAATCGTA
ch2                     TTGCATAAAATTAATTAACATCATCTTTGATCATCTTTAAATCATATGGATTAAATCGTA
VIT_218S0041G01880.2    TTGCATAAAATTAATTAACATCATCTTTGATCATCTTTAAATCATATGGATTAAATCGTA
su_wt                   TTGCATAAAATTAATTAACATCATCTTTGATCATCTTTAAATCATATGGATTAAATCGTA
ch1                     TTGCATAAAATTAATTAACATCATCTTTGATCATCTTTAAATCATATGGATTAAATCGTA
                        ************************************************************


su_mut                  TTGTTGCTTATTTTCCTTATTCAAATAGAATAAAAATTATTTTAAGATTCTTTATTCTTT
su_Di Genova            TTGTTGCTTATTTTCCTTATTCAAATAGAATAAAAATTATTTTAAGATTCTTTATTCTTT
ch2                     TTGTTGCTTATTTTCCTTATTCAAATAGAATAAAAATTATTTTAAGATTCTTTATTCTTT
VIT_218S0041G01880.2    TTGTTGCTTATTTTCCTTATTCAAATAGAATAAAAATTATTTTAAGATTCTTTATTCTTT
su_wt                   TTGTTGCTTATTTTCCTTATTCAAATAGAATAAAAATTATTTTAAGATTCTTTATTCTTT
ch1                     TTGTTGCTTATTTTCCTTATTCAAATAGAATAAAAATTATTTTAAGATTCTTTATTCTTT
                        ************************************************************


su_mut                  ATATATGAGAGAAAAATAAAAGAAATAAATTTGATAAAATATGTGAAAAAATTATTAAGT
su_Di Genova            ATATATGAGAGAAAAATAAAAGAAATAAATTTGATAAAATATGTGAAAAAATTATTAAGT
ch2                     ATATATGAGAGAAAAATAAAAGAAATAAATTTGATAAAATATGTGAAAAAATTATTAAGT
VIT_218S0041G01880.2    ATATATGAGAGAAAAATAGAAGAAATAAATTTGATAAAATATGTGAAAAAATTATTAAGT
su_wt                   ATATATGAGAGAAAAATAAAAGAAATAAATTTGATAAAATATGTGAAAAAATTATTAAGT
ch1                     ATATATGAGAGAAAAATAAAAGAAATAAATTTGATAAAATATGTGAAAAAATTATTAAGT
                        ***************** ******************************************


su_mut                  TCAAGCTTTTTTTATTTATTTTTTATTTTTTCATCATTTTTTTTTTGTTTCTTTTTTTTC
su_Di Genova            TCAAGCTTTTTTTATTTATTTTTTATTTTTTCATCATTTTTTTTTTGTTTCTTTTTTTTC
ch2                     TCAAGCTTTTTTTATTTATTTTTTATTTTTTCATCA-TTTTTTTTTGTTTCTTTTTTTTC
VIT_218S0041G01880.2    TCAAGCTTTTTTTATTTATTTTTTA-CTTTTCATCA-TTTTTTTTTGTTTCTTTTTTTTC
su_wt                   TCAAGCTTTTTTTATTTATTTTTTATTTTTTCATCA-TTTTTTTTTGTTTCTTTTTTTTC
ch1                     TCAAGCTTTTTTTATTTATTTTTTATTTTTTCATCA-TTTTTTTTTGTTTCTTTTTTTTC
                        *********************** ********* *** ******************


su_mut                  TCTCTCTTAAATTTTTGAAGATTTAAATGCAACCCCTACACTTTACACCTAAGAGAGTTT
su_Di Genova            TCTCTCTTAAATTTTYGAAGATTTAAATGCAACCCCTACACTTTACACCTAAGAGAGTTT
ch2                     TCTCTCTTAAATTTTTGAAGATTTAAATGCAACCCCTACACTTTACACCTAAGAGAGTTT
VIT_218S0041G01880.2    TCTCTCTTAAATTTTCGAAGATTTAAATGCAACCCCTACACTTTACACCTAAGAGAGTTT
su_wt                   TCTCTCTTAAATTTTTGAAGATTTAAATGCAACCCCTACACTTTACACCTAAGAGAGTTT
ch1                     TCTCTCTTAAATTTTTGAAGATTTAAATGCAACCCCTACACTTTACACCTAAGAGAGTTT
```

```
                       ***************  ******************************************

su_mut                 GAGGAAAAGTAGGAGAAAAAGAAAACAAAGAAGAAAAATGAAAGAAAGAAAAAGAAAAAT
su_Di Genova           GAGGAAAAGTAGGAGAAAAAGAAAACAAAGAAGAAAAATGAAAGAAAGAAAAAGAAAAAT
ch2                    GAGGAAAAGTAGGAGAAAAAGAAAACAAAGAAGAAAAATGAAAGAAAGAAAAAGAAAAAT
VIT_218S0041G01880.2   GAGGAAAAGTAGGAGAAAAAGAAAACAAAGAAGAAAAATGAAAGAAAGAAAAAGAAAAAT
su_wt                  GAGGAAAAGTAGGAGAAAAAGAAAACAAAGAAGAAAAATGAAAGAAAGAAAAAGAAAAAT
ch1                    GAGGAAAAGTAGGAGAAAAAGAAAACAAAGAAGAAAAATGAAAGAAAGAAAAAGAAAAAT
                       ************************************************************


su_mut                 AAAGAAATATGTTTAAATTCAATAAATTGTTATTACTTATTTTTCTAAAATTATTTTTAT
su_Di Genova           AAAGAAATATGTTTAAATTCAATAAATTGTTATTACTTATTTTTCTAAAATTATTTTTAT
ch2                    AAAGAAATATGTTTAAATTCAATAAATTGTTATTACTTATTTTTCTAAAATTATTTTTAT
VIT_218S0041G01880.2   AAAGAAATATGTTTAAATTCAATAAATTGTTATTACTTATTTTTCTAAAATTATTTTTAT
su_wt                  AAAGAAATATGTTTAAATTCAATAAATTGTTATTACTTATTTTTCTAAAATTATTTTTAT
ch1                    AAAGAAATATGTTTAAATTCAATAAATTGTTATTACTTATTTTTCTAAAATTATTTTTAT
                       ************************************************************


su_mut                 TCTTTTCTCTCATATACAACCAAACAAAATTTCAAAAATGTAAAATTTTTGATATAGTTT
su_Di Genova           TCTTTTCTCTCATATACAACCAAACAAAATTTCAAAAATGTAAAATTTTTGATATAGTTT
ch2                    TCTTTTCTCTCATATACAACCAAACAAAATTTCAAAAATGTAAAATTTTTGATATAGTTT
VIT_218S0041G01880.2   TCTTTTCTCTCATATACAACCAAACAAAATTTCAAAAATGTAAAATTTTTGATATAGTTT
su_wt                  TCTTTTCTCTCATATACAACCAAACAAAATTTCAAAAATGTAAAATTTTTGATATAGTTT
ch1                    TCTTTTCTCTCATATACAACCAAACAAAATTTCAAAAATGTAAAATTTTTGATATAGTTT
                       ************************************************************


su_mut                 TCATTAGATTTGATTTTTCATACTTTCCATAATAATCCAAACAAGATCAATTAATAAACC
su_Di Genova           TCATTAGATTTGATTTTTCATACTTTCCATAATAATCCAAACAAGATCAATTAATAAACY
ch2                    TCATTAGATTTGATTTTTCATACTTTCCATAATAATCCAAACAAGATCAATTAATAAACC
VIT_218S0041G01880.2   TCATTAGATTTGATTTTTCATACTTTCCATAATAATCCAAACAAGATCAATTAATAAACT
su_wt                  TCATTAGATTTGATTTTTCATACTTTCCATAATAATCCAAACAAGATCAATTAATAAACC
ch1                    TCATTAGATTTGATTTTTCATACTTTCCATAATAATCCAAACAAGATCAATTAATAAACC
                       ************************************************************


su_mut                 GAAACAAAACCTACAATGACTTCATAATAACGGAAGTTTAAAGGAACTAAGGATGAATTG
su_Di Genova           GAAACAAAACCTACAATGACTTCATAATAACGGAAGTTTAAAGGAACTAAGGATGAATTG
ch2                    GAAACAAAACCTACAATGACTTCATAATAACGGAAGTTTAAAGGAACTAAGGATGAATTG
VIT_218S0041G01880.2   GAAACAAAACCTACAATGACTTCATAATAACGGAAGTTTAAAGGAACTAAGGATGAATTG
su_wt                  GAAACAAAACCTACAATGACTTCATAATAACGGAAGTTTAAAGGAACTAAGGATGAATTG
ch1                    GAAACAAAACCTACAATGACTTCATAATAACGGAAGTTTAAAGGAACTAAGGATGAATTG
                       ************************************************************


su_mut                 AGATAGATTCGAGGATGGTGTCATAATGATAGGAGTCCAAATTTGGAGGTAAGAATAAAC
su_Di Genova           AGATAGATTCGAGGATGGTGTCATAATGATAGGAGTCCAAATTTGGAGGTAAGAATAAAC
ch2                    AGATAGATTCGAGGATGGTGTCATAATGATAGGAGTCCAAATTTGGAGGTAAGAATAAAC
VIT_218S0041G01880.2   AGATAGATTCGAGGATGGTGTCATAATGATAGGAGTCCAAATTTGGAGGTAAGAATAAAC
su_wt                  AGATAGATTCGAGGATGGTGTCATAATGATAGGAGTCCAAATTTGGAGGTAAGAATAAAC
ch1                    AGATAGATTCGAGGATGGTGTCATAATGATAGGAGTCCAAATTTGGAGGTAAGAATAAAC
                       ************************************************************


su_mut                 TTAAATAAACTCTAAGATGATGCTCCAATGATGAATTTCTTTTTTAAAATATTTGAGTAG
su_Di Genova           TTAAATAAACTCTAAGATGATGCTCCAATGATGAATTTCTTTTTTAAAATATTTGAGTAG
ch2                    TTAAATAAACTCTAAGACGATGCTCCAATGATGAATTTCTTTTTTAAAATATTTGAGTAG
VIT_218S0041G01880.2   TTAAATAAACTCTAAGACGATGCTCCAATGATGAATTTCTTTTTTAAAATATTTGAGTAG
su_wt                  TTAAATAAACTCTAAGACGATGCTCCAATGATGAATTTCTTTTTTAAAATATTTGAGTAG
ch1                    TTAAATAAACTCTAAGACGATGCTCCAATGATGAATTTCTTTTTTAAAATATTTGAGTAG
                       *****************  *****************************************


su_mut                 ACATTTGTCATTAATTTTTCTAACTACAAATAGATTACCAATAAAATGATCATTATCCCT
su_Di Genova           GCATTTGTCATTAATTTTTCTAACTACAAATAGATTACCAATAAAATGATCATAATCCCT
ch2                    GCATTTGTCATTAATTTTTCTAACTACAAATAGATTACCAATAAAATGATCATAATCCCT
VIT_218S0041G01880.2   GCATTTGTCATTAATTTTTCTAACTACAAATAGATTACCAATAAAATGATCATAATCCCT
su_wt                  GCATTTGTCATTAATTTTTCTAACTACAAATAGATTACCAATAAAATGATCATAATCCCT
ch1                    GCATTTGTCATTAATTTTTCTAACTACAAATAGATTACCAATAAAATGATCATAATCCCT
                        *************************************************** ******


su_mut                 TTCTACAGAACTTGTCGATTGTTGTATTGTTTTTTTATGATATTTTTATGTAACATGTTAA
su_Di Genova           TTCAACCGAACTTGTCGGTTGTTGTATTGTTTTTTTATGATATTTTTATGTAACATGTTAA
ch2                    TTCAACCGAACTTGTCGGTTGTTGTATTGTTTTTTTATGATATTTTTATGTAACATGTTAA
```

69

```
VIT_218S0041G01880.2  TTCAACCGAACTTGTCGGTTGTTGTATTGTTTTTTATGATATTTTTATGTAACATGTTAA
su_wt                 TTCAACCGAACTTGTCGGTTGTTGTATTGTTTTTTATGATATTTTTATGTAACATGTTAA
ch1                   TTCAACCGAACTTGTCGGTTGTTGTATTGTTTTTTATGATATTTTTATGTAACATGTTAA
                      *** ** ********** ******************************************


su_mut                ATTATAAAATAAATAAATAAGTTATAAGAAGAAAGAAAAAACCAATTCAATTCCAATAAA
su_Di Genova          ATTATAAAATAAATAAATAAGTTATAAGAAGAAAGAAAAAACCAATTCAATTCCAATAAA
ch2                   ATTATAAAATAAATAAATAAGTTATAAGAAGAAAGAAAAAACCAATTCAATTCCAATAAA
VIT_218S0041G01880.2  ATTATAAAATAAATAAATAAGTTATAAGAAGAAAGAAAAAACCAATTCAATTCCAATAAA
su_wt                 ATTATAAAATAAATAAATAAGTTATAAGAAGAAAGAAAAAACCAATTCAATTCCAATAAA
ch1                   ATTATAAAATAAATAAATAAGTTATAAGAAGAAAGAAAAAACCAATTCAATTCCAATAAA
                      ************************************************************


su_mut                AACCTTTGAAATTAATAAATGGTGGGATATGTG--AGAGGTGCAAAGTCTTGGGTTTGAT
su_Di Genova          AACCTTTGAAATTAATAAATGGTGGGATATGTGATAGAGGTGCAAAGTCTTGGGTTTGAT
ch2                   AACCTTTGAAATTAATAAATGGTGGGATATGTGATAGAGGTGCAAAGTCTTGGGTTCAAT
VIT_218S0041G01880.2  AACCTTTGAAATTAATAAATGGTGGGATATGTGATAGAGGTGCAAAGTCTTGGGTTCAAT
su_wt                 AACCTTTGAAATTAATAAATGGTGGGATATGTGATAGAGGTGCAAAGTCTTGGGTTCAAT
ch1                   AACCTTTGAAATTAATAAATGGTGGGATATGTGATAGAGGTGCAAAGTCTTGGGTTCAAT
                      ********************************  ********************  **


su_mut                TACCATCATTAAGAATCCCTGGATTACTCGGAGTTGATTCTAATGGGTGTCGTTGGAATC
su_Di Genova          TACCATCATTAAGAATCCCTGGATTACTCGGAGTTGATTCTAATGGGTGTCGTTGGAATC
ch2                   TACCATCATTAAGAATCCCTGGATTATTCGGAGTTGATTCTAATGGGTGTCGTTGGAATC
VIT_218S0041G01880.2  TACCATCATTAAGAATCCCTGGATTATTCGGAGTTGATTCTAATGGGTGTCGTTGGAATC
su_wt                 TACCATCATTAAGAATCCCTGGATTATTCGGAGTTGATTCTAATGGGTGTCGTTGGAATC
ch1                   TACCATCATTAAGAATCCCTGGATTATTCGGAGTTGATTCTAATGGGTGTCGTTGGAATC
                      ************************* **********************************


su_mut                CCTAAGGTGTCGAAGCCATGGGTGGATTTGAAAGGCCCTATCACAGTGGGGTTTCCGGGT
su_Di Genova          CCTAAGGTGTCGAAGCCATGGGTGGATTTGAAAGGCCCTATCACAGTGGGGTTTCCGGGT
ch2                   CCTAAGGTGTCGAAGCCATGGGTGGATTTGAAAGGCCCTATCACAGTGGGGTTTCCGGGT
VIT_218S0041G01880.2  CCTAAGGTGTCGAAGCCATGGGTGGATTTGAAAGGCCCTATCACAGTGGGGTTTCCGGTT
su_wt                 CCTAAGGTGTCGAAGCCATGGGTGGATTTGAAAGGCCCTATCACAGTGGGGTTTCCGGTT
ch1                   CCTAAGGTGTCGAAGCCATGGGTGGATTTGAAAGGCCCTATCACAGTGGGGTTTCCGGTT
                      ********************************************************** *


su_mut                TATCAAAAAAAAAAACACACACACACACAATTTTCATAATAGAAGGTAGAAAAAATTGAG
su_Di Genova          TATCAAAAAAAAAAACACACACACACACAATTTTCATAATAGAAGGTAGAAAAAAKTGAG
ch2                   TAT----AAAAAACACACACACACACACAATTTTCATAATAGAAGGTAGAAAAAAGTGAG
VIT_218S0041G01880.2  TAT--AAAAAACACACACACACACACAATTTTCATAATAGAAGGTAGAAAAAAGTGAG
su_wt                 TAT------AAAAAACACACACACACACAATTTTCATAATAGAAGGTAGAAAAAAGTGAG
ch1                   TAT--AAAAAACACACACACACACACACAATTTTCATAATAGAAGGTAGAAAAAAGTGAG
                      ***       ** * ***************************************** ****


su_mut                AATAAGGTAGAGAAGATGTTTTTTCC------TAGGTACTCTAGGGTTTTGTGATAATAG
su_Di Genova          AATAAGGTAGAGAAGATGTTTTTTCCTAGCTATAGGTACTCTAGGGTTTTGTGATAATAG
ch2                   AATAAGGTAGAGAAGATGTTTTTTCCTAGCTATAGGTACTCTAGGGTTTTGTGATAATAG
VIT_218S0041G01880.2  AATAAGGTAGAGAAGATGTTTTTTCCTAGCTATAGGTACTCTAGGGTTTTGTGATAATAG
su_wt                 AATAAGGTAGAGAAGATGTTTTTTCCTAGCTATAGGTACTCTAGGGTTTTGTGATAATAG
ch1                   AATAAGGTAGAGAAGATGTTTTTTCCTAGCTATAGGTACTCTAGGGTTTTGTGATAATAG
                      ************************        **************************


su_mut                ATTTGGATTTTTTTCCCCCAATAATTCAAATTATTAATTCAATTATAATAAATATAAAAA
su_Di Genova          ATTTGGATTTTTTTCCCCCAATAATTCAAATTATTAATTCAATTATAATAAATATAAAAA
ch2                   ATTTGGATTTTTTT-CCCCAATAATTAAAATTATTAATTCAATTATAATAAATATAAAAA
VIT_218S0041G01880.2  ATTTGGATTTTTTT-CCCCAATAATTAAAATTATTAATTCAATTATAATAAATATAAAAA
su_wt                 ATTTGGATTTTTTT-CCCCAATAATTAAAATTATTAATTCAATTATAATAAATATAAAAA
ch1                   ATTTGGATTTTTTT-CCCCAATAATTAAAATTATTAATTCAATTATAATAAATATAAAAA
                      ************* ********** ********************************


su_mut                TCAATGAGAAATTCCATGGAAAACCCAAATCAAATGTTGCAATATAGATCAAGATTAGCA
su_Di Genova          TCAATGAGAAATTCCATGGAAAACCCAAATCAAATGTTGCAATATAGWTCAAGATTAGCA
ch2                   TCAATGAGAAATTCCATGG-AAACCCAAATCAAATGTTGCAATATAGATCAAGATTAGCA
VIT_218S0041G01880.2  TCAATGAGAAATTCCATGG-AAACCCAAATCAAATGTTGCAATATAGATCAAGATTAGCA
su_wt                 TCAATGAGAAATTCCATGG-AAACCCAAATCAAATGTTGCAATATAGTTCAAGATTAGCA
ch1                   TCAATGAGAAATTCCATGG-AAACCCAAATCAAATGTTGCAATATAGATCAAGATTAGCA
                      ******************* ************************** ************
```

```
su_mut               TTTACTTATATGCATGCATGTTAATTAGTTTGACCTTAAAGATCTTGTAGTACTGGTTCC
su_Di Genova         TTTACTTATATGCATGCATGTTAATTAGTTTGACCTTAAAGATCTTGTAGTACTGGTTCC
ch2                  TTTACTTATATGCATGCATGTTAATTAGTTTGACCTTAAAGATCTTGTAGTACTGGTTCC
VIT_218S0041G01880.2 TTTACTTATATGCATGCATGTTAATTAGTTTGACCTTAAAGATCTTGTAGTACTGGTTCC
su_wt                TTTACTTATATGCATGCATGTTAATTAGTTTGACCTTAAAGATCTTGTAGTACTGGTTCC
ch1                  TTTACTTATATGCATGCATGTTAATTAGTTTGACCTTAAAGATCTTGTAGTACTGGTTCC
                     ************************************************************


su_mut               CAATATAAAAATGAGAGCCTATTTGGGATAACTTTTTAACTCCTGCATCTAGTAATTAGA
su_Di Genova         CAATATAAAAATGAGAGCCTATTTGGGATAACTTTTTAACWCCTGCATCTAGTAATTAGA
ch2                  CAATATAAAAATGAGAGCCTATTTGGGATAACTTTTTAACACCTGCATCTAGTAATTAGA
VIT_218S0041G01880.2 CAATATAAAAATGAGAGCCTATTTGGGATAACTTTTTAACACCTGCATCTAGTAATTAGA
su_wt                CAATATAAAAATGAGAGCCTATTTGGGATAACTTTTTAACACCTGCATCTAGTAATTAGA
ch1                  CAATATAAAAATGAGAGCCTATTTGGGATAACTTTTTAACACCTGCATCTAGTAATTAGA
                     *******************************************  ******************


su_mut               GATGAAACAGACATTATTTGTATTATGTAATCTATTTTACGAACAAAATTTTAAAAATGC
su_Di Genova         GATGAAACAGACATTATTTGTATTATGTAATCTATTTTACGAACAAAATTTTAAAAATGC
ch2                  GATGAAACATACATTATTTGTATTATGTAATCTATTTTATGAACAAAATTTTAAAAATGC
VIT_218S0041G01880.2 GATGAAACATACATTATTTGTATTATGTAATCTATTTTATGAACAAAATTTTAAAAATGC
su_wt                GATGAAACATACATTATTTGTATTATGTAATCTATTTTATGAACAAAATTTTAAAAATGC
ch1                  GATGAAACATACATTATTTGTATTATGTAATCTATTTTATGAACAAAATTTTAAAAATGC
                     ********* ********************** ********  ******************


su_mut               TTTAATAAGTAGTGAGGATGACTATGATATTTCAAAAAAAATTATTAATTAAAAAAATTT
su_Di Genova         TTTAATAAATAGTGAGGATGACTATGATATTTCAAAAAAAATTATTAATTAAAAAAATTT
ch2                  TTTAATAAATAGTGAGGATGACTATGATATTTCA---------------AAAAAATTTT
VIT_218S0041G01880.2 TTTAATAAATAGTGAGGATGACTATGATATTTCA---------------AAAAAATTTT
su_wt                TTTAATAAATAGTGAGGATGACTATGATATTTCA---------------AAAAAATTTT
ch1                  TTTAATAAATAGTGAGGATGACTATGATATTTCA---------------AAAAAATTTT
                     ********  ***********************         *****  ***


su_mut               ACTAACAAAAATGTCGATAAATCATTTATAAGTGTTATCTACATGTCCTTAAAATCACTT
su_Di Genova         ACTAACAAAAATGTCGATAAATCATTTATAAGTGTTATCTAAATGCCCTTAAAATCACTT
ch2                  ACTAACAAAAATGTCGATAAATCATTTATAAGTGTTATCTAAATGCCCTTAAAATCACTT
VIT_218S0041G01880.2 ACTAACAAAAATGTCGATAAATCATTTATAAGTGTTATCTAAATGCCCTTAAAATCACTT
su_wt                ACTAACAAAAATGTCGATAAATCATTTATAAGTGTTATCTAAATGCCCTTAAAATCACTT
ch1                  ACTAACAAAAATGTCGATAAATCATTTATAAGTGTTATCTAAATGCCCTTAAAATCACTT
                     *****************************************  *** *************


su_mut               ATTTAAATAATAGAAATTAAGCTAGATTAATAATGAATTAAAAGAAAA-----AAAAATT
su_Di Genova         ATTTAAATAATAGAAATTAAGCTAGATTAATAATGAATGAAAAGAAAAAAAATAAAATTT
ch2                  ATTTAAATAATAGAAATTAAGCTAGATTAATAATGAATGAAAAGAAAAAAAATAAAATTT
VIT_218S0041G01880.2 ATTTAAATAATAGAAATTAAGCTAGATTAATAATGAATGAAAAGAAAAAAAATAAAATTT
su_wt                ATTTAAATAATAGAAATTAAGCTAGATTAATAATGAATGAAAAGAAAAAAAATAAAATTT
ch1                  ATTTAAATAATAGAAATTAAGCTAGATTAATAATGAATGAAAAGAAAAAAAATAAAATTT
                     **************************************** ********     **** **


su_mut               GTACAAACCATCCAGTTTAGTACTGGTCCGAACCCCCTTCTAGCTCAAAAGGTCAATAGG
su_Di Genova         GTACAAATCATCCAGTTTAGTACTGGTCGGAACCCCCTTCAAGCTCAAAAGGTCAATCGG
ch2                  GTACAAATCATCCAGTTTAGTACTGGTCGGAACCCCCTTCAAGCTCAAAAGGTCAATCGG
VIT_218S0041G01880.2 GTACAAATCATCCAGTTTAGTACTGGTCGGAACCCCCTTCAAGCTCAAAAGGTCAATCGG
su_wt                GTACAAATCATCCAGTTTAGTACTGGTCGGAACCCCCTTCAAGCTCAAAAGGTCAATCGG
ch1                  GTACAAATCATCCAGTTTAGTACTGGTCGGAACCCCCTTCAAGCTCAAAAGGTCAATCGG
                     *******  ****************** ************  ************* **


su_mut               ATTGGACTAGGATCCGGTCAATAGTTCGATTGATTAAATCGATCAATT-GGTCCGATGTT
su_Di Genova         ATTGGACTAGGATCTGGTCAACAGTTCGATTGATT-----GATCAATTCGGTCCGATCTT
ch2                  ATTGGACTAGGATCTGGTCAACAGTTCGATTGATT-----GATCAATTCGGTCCGATCTT
VIT_218S0041G01880.2 ATTGGACTAGGATCTGGTCAACAGTTCGATTGATT-----GATCAATTCGGTCCGATCTT
su_wt                ATTGGACTAGGATCTGGTCAACAGTTCGATTGATT-----GATCAATTCGGTCCGATCTT
ch1                  ATTGGACTAGGATCTGGTCAACAGTTCGATTGATT-----GATCAATTCGGTCCGATCTT
                     ************** ****** *************        ******** ******** **


su_mut               TAAAACATTACAAATAACTTATCTTATCTGAGTTTTGATGCATCTCATACATGCAAAGTA
su_Di Genova         TAAAACATTACAAATAACTTATCTTATGTGAGTTTTGGTGCATCTCATACATGCAAAGCA
ch2                  TAAAACATTACAAATAACTTATCTTATGTGAGTTTTGGTGCATCTCATTCATGCAAAGCA
VIT_218S0041G01880.2 TAAAACATTACAAATAACTTATCTTATGTGAGTTTTGGTGCATCTCATACATGCAAAGCA
su_wt                TAAAACATTACAAATAACTTATCTTATGTGAGTTTTGGTGCATCTCATACATGCAAAGCA
ch1                  TAAAACATTACAAATAACTTATCTTATGTGAGTTTTGGTGCATCTCATTCATGCAAAGCA
```

```
                       *************************** ********* ********** ********* *

su_mut                 GTTATTGAAAGCCAATACATATTTCATACAAAGTCCACACAAAGAAACTCAAATAAAAAA
su_Di Genova           GTTATTGAAAGCCAATACATATTTCATACAAAGTCCACACAAAGAAACTCAAATAAAAAA
ch2                    GTTATTGAAAGCCAATACATATTTCATACAAAGTCCACACAAAGAAACTCAAATAAAAAA
VIT_218S0041G01880.2   GTTATTGAAAGCCAATACATATTTCATACAAAGTCCACACAAAGAAACTCAAATAAAAAA
su_wt                  GTTATTGAAAGCCAATACATATTTCATACAAAGTCCACACAAAGAAACTCAAATAAAAAA
ch1                    GTTATTGAAAGCCAATACATATTTCATACAAAGTCCACACAAAGAAACTCAAATAAAAAA
                       ************************************************************


su_mut                 ATAAAAAAATCAGAGAATAAAATCCTCATGGTAAAGTTGCACCAATAAGACCTATACTGC
su_Di Genova           ATAAAAAAATCAGAGAATAAAATCCTCATGGTAAAGTTGCACCAATAAGACCTATACTGC
ch2                    ATAAAAAAATCAGAGAATAAAATCCTCATGGTAAAGTTGCACCAATAAGACCTATACTGC
VIT_218S0041G01880.2   ATAAAAAAATCAGAGAATAAAATCCTCATGGTAAAGTTGCACCAATAAGACCTATACTGC
su_wt                  ATAAAAAAATCAGAGAATAAAATCCTCATGGTAAAGTTGCACCAATAAGACCTATACTGC
ch1                    ATAAAAAAATCAGAGAATAAAATCCTCATGGTAAAGTTGCACCAATAAGACCTATACTGC
                       ************************************************************


su_mut                 ATACAGTTGCAGAGTGGCAGTGCTCTCCATTGTTCTTTCTCTTGCTTTTCAGTGGTGCAA
su_Di Genova           ATACAGTTGCAGAGTGGCAGTGCTCTYCATTGTTCTTTCTCTTGCTTTTCAGTGGTGCAA
ch2                    ATACAGTTGCAGAGTGGCAGTGCTCTTCATTGTTCTTTCTCTTGCTTTTCAGTGGTGTAA
VIT_218S0041G01880.2   ATACAGTTGCAGAGTGGCAGTGCTCTTCATTGTTCTTTCTCTTGCTTTTCAGTGGTGTAA
su_wt                  ATACAGTTGCAGAGTGGCAGTGCTCTTCATTGTTCTTTCTCTTGCTTTTCAGTGGTGTAA
ch1                    ATACAGTTGCAGAGTGGCAGTGCTCTTCATTGTTCTTTCTCTTGCTTTTCAGTGGTGTAA
                       *************************  *****************************  **


su_mut                 TCCATGAGAATCTGAACCCTCTGGCAGTGTCTGAAAAAGGGTGGTCATAGCAGTACAACC
su_Di Genova           TCCATGAGAATCTGAACCCTCTGGCAGTGTCTGAAAAAGGGTGGTCATAGCAGTACAACC
ch2                    TCCATGAGAATCTGAACCATCTGGCAGTGTCTGAAAAAGGGAGGTCATAGCAGTACAACC
VIT_218S0041G01880.2   TCCATGAGAATCTGAACCATCTGGCAGTGTCTGAAAAAGGGAGGTCATAGCAGTACAACC
su_wt                  TCCATGAGAATCTGAACCATCTGGCAGTGTCTGAAAAAGGGAGGTCATAGCAGTACAACC
ch1                    TCCATGAGAATCTGAACCATCTGGCAGTGTCTGAAAAAGGGAGGTCATAGCAGTACAACC
                       ******************  ********************  ******************


su_mut                 AACCACTCATTTTTCATCTTTCCTTGTATCTTCCCTTGTTTGCAAAATCTCAGTTTTTCT
su_Di Genova           AACCACTCATTTTTCATCTTTCCTTGTATCTTCCCTTGTTTGCAAAATCTCAGTTTTTCT
ch2                    AACCACTCATTTTTCATCTTTCCTTGTATCTTCCCTTGTTTGCAAAATCTCAGTTTTTCT
VIT_218S0041G01880.2   AACCACTCATTTTTCATCTTTCCTTGTATCTTCCCTTGTTTGCAAAATCTCAGTTTTTCT
su_wt                  AACCACTCATTTTTCATCTTTCCTTGTATCTTCCCTTGTTTGCAAAATCTCAGTTTTTCT
ch1                    AACCACTCATTTTTCATCTTTCCTTGTATCTTCCCTTGTTTGCAAAATCTCAGTTTTTCT
                       ************************************************************


su_mut                 GGTTGTTTCTTTCCACTTTTGGCTTTCCGCAAACTTTGAATGGAGTCTTGGGTCTTTTCT
su_Di Genova           GGTTGTTTCTTTCCACTTTTGGCTTTCCGCAAACTTTGAATGGAGTCTTGGGTCTTTTCT
ch2                    GGTTGTTTCTTTCCACTTTTGGCTTTCCGCAAACTTTGAATGGAGTCTTGGGTCTTTTCT
VIT_218S0041G01880.2   GGTTGTTTCTTTCCACTTTTGGCTTTCCGCAAACTTTGAATGGAGTCTTGGGTCTTTTCT
su_wt                  GGTTGTTTCTTTCCACTTTTGGCTTTCCGCAAACTTTGAATGGAGTCTTGGGTCTTTTCT
ch1                    GGTTGTTTCTTTCCACTTTTGGCTTTCCGCAAACTTTGAATGGAGTCTTGGGTCTTTTCT
                       ************************************************************


su_mut                 CTTTGCCTCTCAAAGCTGCAATCACTGCTCTTTTTTAGTGCTGGAGAGTAGAAACCAAGA
su_Di Genova           CTTTGCCTCTCAAAGCTGCAATCACTGCTCTTTTTTAGTGCTGGAGAGTAGAAACCAAGA
ch2                    CTTTGCCTCTCAAAGCTGCAATCACTGCTCTTTTTTAGTGCTGGAGAGGAGAAACCAGGA
VIT_218S0041G01880.2   CTTTGCCTCTCAAAGCTGCAATCACTGCTCTTTTTTAGTGCTGGAGAGGAGAAACCAGGA
su_wt                  CTTTGCCTCTCAAAGCTGCAATCACTGCTCTTTTTTAGTGCTGGAGAGGAGAAACCAGGA
ch1                    CTTTGCCTCTCAAAGCTGCAATCACTGCTCTTTTTTAGTGCTGGAGAGGAGAAACCAGGA
                       ************************************************ ********  **


su_mut                 CAAGACTTTTCAACTGCTATTCTCCATACAAACTCATGAAACTGATGAACAATTGAGCAG
su_Di Genova           CAAGACTTTTCAACTGCTATTCTCCATACAAACTCATGAAACTGATGAACAATTGAGCAG
ch2                    CAAGACTTTTCAACTGCTATTCTCCATACAAACTCATGAAACTGATGAACAATTGAGCAG
VIT_218S0041G01880.2   CAAGACTTTTCAACTGCTATTCTCCATACAAACTCATGAAACTGATGAACAATTGAGCAG
su_wt                  CAAGACTTTTCAACTGCTATTCTCCATACAAACTCATGAAACTGATGAACAATTGAGCAG
ch1                    CAAGACTTTTCAACTGCTATTCTCCATACAAACTCATGAAACTGATGAACAATTGAGCAG
                       ************************************************************
```

```
su_mut              TAGGGTCACTGTGCATACTGTATAGTGATTTTAATCATTTTTATGTCTCATAACTTGTGG
su_Di Genova        TAGGGTCACTGTGYATACTGTATAGTGATTTTAATCATTTTTATGTCTCATAACTTGTGG
ch2                 TAGGGTCACTGTGTATACTGTATAGTGATTTTAATCATTTTTATGTCTCATAACTTGTGG
VIT_218S0041G01880.2 TAGGGTCACTGTGTATACTGTATAGTGATTTTAATCATTTTTATGTCTCATAACTTGTGG
su_wt               TAGGGTCACTGTGTATACTGTATAGTGATTTTAATCATTTTTATGTCTCATAACTTGTGG
ch1                 TAGGGTCACTGTGTATACTGTATAGTGATTTTAATCATTTTTATGTCTCATAACTTGTGG
                    ************** ********************************************

su_mut              GTGTTTGTACTGTTACTTCATCTTTCTCATATCAAGTCATAGTCCTTTCACAAACTTGTG
su_Di Genova        GTGTTTGTACTGTTACTTCATCTTTCTCATATCAAGTCATAGTCCTTTCACAAACTTGTG
ch2                 GTGTTTGTACTGTTACTTCATCTTTCTCATATCAAGTCATAGTCCTTTCACAAACTTGTG
VIT_218S0041G01880.2 GTGTTTGTACTGTTACTTCATCTTTCTCATATCAAGTCATAGTCCTTTCACAAACTTGTG
su_wt               GTGTTTGTACTGTTACTTCATCTTTCTCATATCAAGTCATAGTCCTTTCACAAACTTGTG
ch1                 GTGTTTGTACTGTTACTTCATCTTTCTCATATCAAGTCATAGTCCTTTCACAAACTTGTG
                    ************************************************************

su_mut              CTGTCATCTCCCATCATATATGGTTTCCTCCTTTAGGGTTTTGCTTTCCGCCTTCACTTT
su_Di Genova        CTGTCATCTCCCATCATATATGGTTTCCTCCTTTAGGGTTTTGCTTTCCGCCTTCACTTT
ch2                 CTGTCATCTCCCATCATATATGGTTTCCTCCTTTAGGGTTTTGCTTTCCGCCTTCACTTT
VIT_218S0041G01880.2 CTGTCATCTCCCATCATATATGGTTTCCTCCTTTAGGGTTTTGCTTTCCGCCTTCACTTT
su_wt               CTGTCATCTCCCATCATATATGGTTTCCTCCTTTAGGGTTTTGCTTTCCGCCTTCACTTT
ch1                 CTGTCATCTCCCATCATATATGGTTTCCTCCTTTAGGGTTTTGCTTTCCGCCTTCACTTT
                    ************************************************************

su_mut              TGACTACTTTTGGAGCAATTTTCTCTCTTGAATTCTATCATATGTAATATTTCTATCCAT
su_Di Genova        TGACTACTTTTGGAGCAATTTTCTCTCTTGAATTCTATCATATGTAATATTTCTATCCAT
ch2                 TGACTACTTTTGGAGCAATTTTCTCTCTTGAATTCTATGGTATGTAATATTTCAATCCAT
VIT_218S0041G01880.2 TGACTACTTTTGGAGCAATTTTCTCTCTTGAATTCTATGGTATGTAATATTTCAATCCAT
su_wt               TGACTACTTTTGGAGCAATTTTCTCTCTTGAATTCTATGGTATGTAATATTTCAATCCAT
ch1                 TGACTACTTTTGGAGCAATTTTCTCTCTTGAATTCTATGGTATGTAATATTTCAATCCAT
                    **************************************  ************ ******

su_mut              TTTGCAACAATTTTTTTAATTCATTCTATTTTTATATATTGGAAAACAGCATAAAATCAA
su_Di Genova        TTTGCAACAATTTTTTTAATTCATTCTATTTTTATATATTGGAAAACAGCATAAAATCAA
ch2                 TTTGCAACAATTTTTTTAATTCATTCTATTTTTATATATTGGAAAACAGCATAAAATCAA
VIT_218S0041G01880.2 TTTGCAACAATTTTTTTAATTCATTCTATTTTTATATATTGGAAAACAGCATAAAATCAA
su_wt               TTTGCAACAATTTTTTTAATTCATTCTATTTTTATATATTGGAAAACAGCATAAAATCAA
ch1                 TTTGCAACAATTTTTTTAATTCATTCTATTTTTATATATTGGAAAACAGCATAAAATCAA
                    ************************************************************

su_mut              CCATAGATAGGTACAAGAAGGCCAGCTCAGATAGTACAAATGGAGGCTCTACCATGGAGA
su_Di Genova        CCATAGATAGGTACAAGAAGGCCAGCTCAGATAGTACAAATGGAGGCTCTACCATGGAGA
ch2                 CCATAGATAGGTACAAGAAGGCCAGCTCAGATAGTACAAATGGAGGCTCTACCATGGAGA
VIT_218S0041G01880.2 CCATAGATAGGTACAAGAAGGCCAGCTCAGATAGTACAAATGGAGGCTCTACCATGGAGA
su_wt               CCATAGATAGGTACAAGAAGGCCAGCTCAGATAGTACAAATGGAGGCTCTACCATGGAGA
ch1                 CCATAGATAGGTACAAGAAGGCCAGCTCAGATAGTACAAATGGAGGCTCTACCATGGAGA
                    ************************************************************

su_mut              TCAATGCCCAAGTAAGAAAACTTCCATTTTATGATAGTTAATGAATCAATCCAAATCCTA
su_Di Genova        TCAATGCCCAAGTAAGAAAACTTCCATTTTATGATAGTTAATGAATCAATCCAAATCCTA
ch2                 TCAATGCCCAAGTAAGAAAACTTCCATTTTATGATAGTTAATGAATCAATCCAAATCCTA
VIT_218S0041G01880.2 TCAATGCCCAAGTAAGAAAACTTCCATTTTATGATAGTTAATGAATCAATCCAAATCCTA
su_wt               TCAATGCCCAAGTAAGAAAACTTCCATTTTATGATAGTTAATGAATCAATCCAAATCCTA
ch1                 TCAATGCCCAAGTAAGAAAACTTCCATTTTATGATAGTTAATGAATCAATCCAAATCCTA
                    ************************************************************

su_mut              TATGGTTTACTGATCAGATGTCTTATCCACCATGGCTGTTAGAACATCTCAATCCATTCT
su_Di Genova        TATGGTTTACTGATCAGATGTCTTATCCACCATGGCTGTTAGAACATCTCAATCCATTCT
ch2                 TATGGTTTACTGATCAGATGTCTTATCCACCATGGCTGTTAGAACATCTCAATCCATTCT
VIT_218S0041G01880.2 TATGGTTTACTGATCAGATGTCTTATCCACCATGGCTGTTAGAACATCTCAATCCATTCT
su_wt               TATGGTTTACTGATCAGATGTCTTATCCACCATGGCTGTTAGAACATCTCAATCCATTCT
ch1                 TATGGTTTACTGATCAGATGTCTTATCCACCATGGCTGTTAGAACATCTCAATCCATTCT
                    ************************************************************

su_mut              GTGGATTGAAAAGTGCACATTTTTCATTAGGCTGGCCACAAATTAAAACCCTCATCATGC
su_Di Genova        GTGGATTGAAAAGTGCACATTTTTCATTAGGCTGGCCACAAATTAAAACCCTCATCATGC
ch2                 GTGGATTGAAAAGTGCACATTTTTCATTAGGCTGGCCACAAATTAAAACCCTCATCATGC
VIT_218S0041G01880.2 GTGGATTGAAAAGTGCACATTTTTCATTAGGCTGGCCACAAATTAAAACCCTCATCATGC
su_wt               GTGGATTGAAAAGTGCACATTTTTCATTAGGCTGGCCACAAATTAAAACCCTCATCATGC
ch1                 GTGGATTGAAAAGTGCACATTTTTCATTAGGCTGGCCACAAATTAAAACCCTCATCATGC
```

```
                        ************************************************************
su_mut                  TTAGCCATAAACAAATTTGTTAAAATCATTATTCTACTTTCTTTTTTCTTTTTGGATTAT
su_Di Genova            TTAGCCATAAACAAATATGTTAAAATCATTATCCTACTTTCTTTTTTCTTTTTGGATTAT
ch2                     TTAGCCATAAACAAATATGTTAAAATCATTATCCTACTTTCTTTTTTCTTTTTGGATTAT
VIT_218S0041G01880.2    TTAGCCATAAACAAATATGTTAAAATCATTATCCTACTTTCTTTTTTCTTTTTGGATTAT
su_wt                   TTAGCCATAAACAAATATGTTAAAATCATTATCCTACTTTCTTTTTTCTTTTTGGATTAT
ch1                     TTAGCCATAAACAAATATGTTAAAATCATTATCCTACTTTCTTTTTTCTTTTTGGATTAT
                        *************** *************** **************************

su_mut                  CCCATTGAACTTTCTCGAATTCTGTCGTTGAACATAGTATTACCAGCAAGAATCAGCAAA
su_Di Genova            CCCATTGAACTTTCTCGAATTCTGTTGTTGAACATAGTACTACCAGCAAGAATCAGCAAA
ch2                     CCCATTGAACTTTCTCGAATTCTGTTGTTGAACATAGTACTACCAGCAAGAATCAGCAAA
VIT_218S0041G01880.2    CCCATTGAACTTTCTCGAATTCTGTTGTTGAACATAGTACTACCAGCAAGAATCAGCAAA
su_wt                   CCCATTGAACTTTCTCGAATTCTGTTGTTGAACATAGTACTACCAGCAAGAATCAGCAAA
ch1                     CCCATTGAACTTTCTCGAATTCTGTTGTTGAACATAGTACTACCAGCAAGAATCAGCAAA
                        ************************* ************* *******************

su_mut                  GCTGCGCCAGCAAATACAGATGCTGCAGAATTCTAACAGGTACCCTTGATTCATATTTCA
su_Di Genova            GCTGCGCCAGCAAATACAGATGCTGCAGAATTCTAACAGGTACCCTTGATTCATATTTCA
ch2                     GCTGCGCCAGCAAATACAGATGCTGCAGAATTCTAACAGGTACCCTTGATTCATATTTCA
VIT_218S0041G01880.2    GCTGCGCCAGCAAATACAGATGCTGCAGAATTCTAACAGGTACCCTTGATTCATATTTCA
su_wt                   GCTGCGCCAGCAAATACAGATGCTGCAGAATTCTAACAGGTACCCTTGATTCATATTTCA
ch1                     GCTGCGCCAGCAAATACAGATGCTGCAGAATTCTAACAGGTACCCTTGATTCATATTTCA
                        ************************************************************

su_mut                  TTTTTCTCACATGTAATTGAGTGTATATATATATGTCAATTTGCATTTTCCTTCTGCTTG
su_Di Genova            TTTTTCTCACATGCAGTTGAGTGTATATATATATGTCAATTTGCATTTTCCTTCTGCTTG
ch2                     TTTTTCTCACATGCAGTTGAGTGTATATATATATGTCAATTTGCATTTTCCTTCTGCTTG
VIT_218S0041G01880.2    TTTTTCTCACATGCAGTTGAGTGTATATATATATGTCAATTTGCATTTTCCTTCTGCTTG
su_wt                   TTTTTCTCACATGCAGTTGAGTGTATATATATATGTCAATTTGCATTTTCCTTCTGCTTG
ch1                     TTTTTCTCACATGCAGTTGAGTGTATATATATATGTCAATTTGCATTTTCCTTCTGCTTG
                        ************* * ********************************************

su_mut                  TGTCTTTAACTAGGTTTTTGGCATCAGTTCCCATGCAAAAATTTCAAATATTACTTAACG
su_Di Genova            TGTCTTTAACTAGGTTTTTGRCATCAGTTCCCATGCAAAAATTTCAAATATTACTTAACG
ch2                     TGTCTTTAACTAGGTTTTTGACATCAGTTCCCATGCAAAAATTTCAAATATTACCTAACG
VIT_218S0041G01880.2    TGTCTTTAACTAGGTTTTTGACATCAGTTCCCATGCAAAAATTTCAAATATTACTTAACG
su_wt                   TGTCTTTAACTAGGTTTTTGACATCAGTTCCCATGCAAAAATTTCAAATATTACTTAACG
ch1                     TGTCTTTAACTAGGTTTTTGACATCAGTTCCCATGCAAAAATTTCAAATATTACTTAACG
                        ******************* ***************************** *****

su_mut                  ATCTTAATTAGGTATGTTAAGACTTAAGAGCAGGTTATACTAAGACATTTTGTCTGTGTT
su_Di Genova            ATCTTAATTAGATATGTTAAGACTTAAGATCAGGTTGTACTAAGACATTTTGTCTGTGTT
ch2                     ATCTTAATTAGATATGTTAAGACTTAAGATCAGGTTGTACTAAGACATTTTGTCTGTGTT
VIT_218S0041G01880.2    ATCTTAATTAGATATGTTAAGACTTAAGATCAGGTTGTACTAAGACATTTTGTCTGTGTT
su_wt                   ATCTTAATTAGATATGTTAAGACTTAAGATCAGGTTGTACTAAGACATTTTGTCTGTGTT
ch1                     ATCTTAATTAGATATGTTAAGACTTAAGATCAGGTTGTACTAAGACATTTTGTCTGTGTT
                        *********** **************** ****** **********************

su_mut                  TATAGGCACTTAATGGGTGATTCCTTGGCTTCCTTGACTGTGAAGGAGCTAAAGCAGCTC
su_Di Genova            TATAGGCACTTAATGGGTGATTCCTTGGCTTCCTTGACTGTGAAGGAGCTAAAGCAGCTC
ch2                     TATAGGCACTTAATGGGTGATTCCTTGGCTTCCTTGACTGTGAAGGAGCTAAAGCAGCTC
VIT_218S0041G01880.2    TATAGGCACTTAATGGGTGATTCCTTGGCTTCCTTGACTGTGAAGGAGCTAAAGCAGCTC
su_wt                   TATAGGCACTTAATGGGTGATTCCTTGGCTTCCTTGACTGTGAAGGAGCTAAAGCAGCTC
ch1                     TATAGGCACTTAATGGGTGATTCCTTGGCTTCCTTGACTGTGAAGGAGCTAAAGCAGCTC
                        ************************************************************

su_mut                  GAGAACAGGCTTGAACGAGGCATCACAAGAATCAGGTCGAAGAAGGTAACGACAC--ATA
su_Di Genova            GAGAACAGGCTTGAACGAGGCATCACAAGAATCAGGTCGAAGAAGGTAACGACAC--ATA
ch2                     GAGAACAGGCTTGAACGAGGCATCACAAGAATCAGGTCGAAGAAGGTAACGACACCTAGA
VIT_218S0041G01880.2    GAGAACAGGCTTGAACGAGGCATCACAAGAATCAGGTCGAAGAAGGTAACGACACCTAGA
su_wt                   GAGAACAGGCTTGAACGAGGCATCACAAGAATCAGGTCGAAGAAGGTAACGACACCTAGA
ch1                     GAGAACAGGCTTGAACGAGGCATCACAAGAATCAGGTCGAAGAAGGTAACGACACCTAGA
                        ***************************************************** * *

su_mut                  CACTAATTCTAATCTGGCTGGGCATTTGACTTTGTGACTTAAATGAAATTGAAAAAAAAA
su_Di Genova            CACTAATTCTAATCTGGCTGGGCATTTGACTTTGTGACTTAAATGAAATTGAAAAAAAAA
ch2                     CACTAAATCTAATCTGGCTGAGCATTTGATTTTGAGACTTAAATGAAA-TGGAAAAAAAA
```

```
VIT_218S0041G01880.2 CACTAAATCTAATCTGGCTGAGCATTTGATTTTGAGACTTAAATGAAA-TGGAAAAAAAA
su_wt                CACTAAATCTAATCTGGCTGAGCATTTGATTTTGAGACTTAAATGAAA-TGGAAAAAAAA
ch1                  CACTAAATCTAATCTGGCTGAGCATTTGATTTTGAGACTTAAATGAAA-TGGAAAAAAAA
                     ****** ************* ******** **** ************ ** ********


su_mut               ATGGTATCATTCACTGATTA---AATGATACATGAAGATTGAATTAATTTACGATACTGC
su_Di Genova         ATGGTATCATTCACTGATTAAATAATAATAATACTTGAAGGTTGAATTAATTTAAGATACCGC
ch2                  ATGGTATCATTCACTGATTAAATAATAATAATACTTGAAGGTTGAATTAATTTAAGATACCGC
VIT_218S0041G01880.2 ATGGTATCATTCACTGATTAAATAATAATAATACTTGAAGGTTGAATTAATTTAAGATACCGC
su_wt                ATGGTATCATTCACTGATTAAATAATAATAATACTTGAAGGTTGAATTAATTTAAGATACCGC
ch1                  ATGGTATCATTCACTGATTAAATAATAATAATACTTGAAGGTTGAATTAATTTAAGATACCGC
                     *******************    *** **** ***** ************* ***** **


su_mut               TTTCACTTGATAACTAAAGCCTGATGGTAGGAACTTCCACCCATCTAGCTTAGTTGGTGA
su_Di Genova         TTTCACTTGATAACTAAAGCCTGATGGTAGGAACTTCCACCCATCTAGCTTAGTTGGTGA
ch2                  TTTCACTTGATAACTAAAGCCTGATGGTAGGAACTTCCACCCATCTAGCTTAGTTGGTGA
VIT_218S0041G01880.2 TTTCACTTGATAACTAAAGCCTGATGGTAGGAACTTCCACCCATCTAGCTTAGTTGGTGA
su_wt                TTTCACTTGATAACTAAAGCCTGATGGTAGGAACTTCCACCCATCTAGCTTAGTTGGTGA
ch1                  TTTCACTTGATAACTAAAGCCTGATGGTAGGAACTTCCACCCATCTAGCTTAGTTGGTGA
                     ************************************************************


su_mut               TTGAGCTGAATTTTGTCTTGGAAACTTTGTGAAAAATAGACAAGTGATCACTAGATCAGC
su_Di Genova         TTGAGCTGAATTTTGTCTTGGAAACTTTGTGAAAAATAGACAAGTGATCACTAGATCAGC
ch2                  TTGAGCTGAATTTTGTCTTGGAAACTTTGTGAAAAATAGACAAGTGATCACTAGATCAGC
VIT_218S0041G01880.2 TTGAGCTGAATTTTGTCTTGGAAACTTTGTGAAAAATAGACAAGTGATCACTAGATCAGC
su_wt                TTGAGCTGAATTTTGTCTTGGAAACTTTGTGAAAAATAGACAAGTGATCACTAGATCAGC
ch1                  TTGAGCTGAATTTTGTCTTGGAAACTTTGTGAAAAATAGACAAGTGATCACTAGATCAGC
                     ************************************************************


su_mut               AAAGCCTATCTTTTTTCCTTTGTTCTAATGTTGTGCTTGTTCTAATGCAATAGCATGAGT
su_Di Genova         AAAGCCTATCTTTTTTCCTTTGTTCTAATGTCGTGCTTGTTCTTATGCAATAGCATGAGT
ch2                  AAAGCCTATCTTTTTTCCTTTGTTCTAATGTCGTGCTTGTTCTTATGCAATAGCATGAGT
VIT_218S0041G01880.2 AAAGCCTATCTTTTTTCCTTTGTTCTAATGTCGTGCTTGTTCTTATGCAATAGCATGAGT
su_wt                AAAGCCTATCTTTTTTCCTTTGTTCTAATGTCGTGCTTGTTCTTATGCAATAGCATGAGT
ch1                  AAAGCCTATCTTTTTTCCTTTGTTCTAATGTCGTGCTTGTTCTTATGCAATAGCATGAGT
                     ****************************** ********** ****************


su_mut               TGCTGTTGGCTGAGATTGAATACTTGCAGAAAAGGGTAATCTCTTGTTCTAAACATCAAT
su_Di Genova         TGCTGTTGGCTGAGATTGAGTACTTGCAGAAAAGGGTAATCTCTTGTTCTAAACATCAAT
ch2                  TGCTGTTGGCTGAGATTGAGTACTTGCAGAAAAGGGTAATCTCTTGTTCTAAACATCAAT
VIT_218S0041G01880.2 TGCTGTTGGCTGAGATTGAGTACTTGCAGAAAAGGGTAATCTCTTGTTCTAAACATCAAT
su_wt                TGCTGTTGGCTGAGATTGAGTACTTGCAGAAAAGGGTAATCTCTTGTTCTAAACATCAAT
ch1                  TGCTGTTGGCTGAGATTGAGTACTTGCAGAAAAGGGTAATCTCTTGTTCTAAACATCAAT
                     ***************** ******************************************


su_mut               TTCCATGTTGGTTCCCTTCTTTTATGATTTTACCAGAATTCTTTATCGAGCATGTGTTAA
su_Di Genova         TTCCATGTKGGTTCCCTTCTTTTATGATTTTTCCAGAATTCTTTATCGAGCACCTTTTAA
ch2                  TTCCATGTGGGTTCCCTTCTTTTATGATTTTTCCAGAATTCTTTATCGAGCACCTTTTAA
VIT_218S0041G01880.2 TTCCATGTGGGTTCCCTTCTTTTATGATTTTTCCAGAATTCTTTATCGAGCACCTTTTAA
su_wt                TTCCATGTGGGTTCCCTTCTTTTATGATTTTTCCAGAATTCTTTATCGAGCACCTTTTAA
ch1                  TTCCATGTGGGTTCCCTTCTTTTATGATTTTTCCAGAATTCTTTATCGAGCACCTTTTAA
                     ******** ******************** ******************* * ****


su_mut               TTTTCTTAGGAAATTGAGCTGGAAAATGAAAGCGTATATCTCCGAACCAAGGTATGAATT
su_Di Genova         TTTTCTTAGGAAATTGAGCTGGAAAATGAAAGCGTATATCTCCGAACCAAGGTATGAATT
ch2                  TTTTCTTAGGAAATTGAGCTGGAAAATGAAAGCGTATATCTCCGAACCAAGGTATGAATT
VIT_218S0041G01880.2 TTTTCTTAGGAAATTGAGCTGGAAAATGAAAGCGTATATCTCCGAACCAAGGTATGAATT
su_wt                TTTTCTTAGGAAATTGAGCTGGAAAATGAAAGCGTATATCTCCGAACCAAGGTATGAATT
ch1                  TTTTCTTAGGAAATTGAGCTGGAAAATGAAAGCGTATATCTCCGAACCAAGGTATGAATT
                     ************************************************************


su_mut               AAATTAGTATAGGAATTGAATGTGATAAAATTGAAGGCACAACAGTTGATACCGATCTAG
su_Di Genova         AAATTAGTATAGGAATTGAATGTGATAAAATTGAAGGCACAACAGTTGATACCGATCTAG
ch2                  AAATTAGTATAGGAATTGAATGTGATAAAATTGAAGGCACAACAGTTGATACCGATCTAG
VIT_218S0041G01880.2 AAATTAGTATAGGAATTGAATGTGATAAAATTGAAGGCACAACAGTTGATACCGATCTAG
su_wt                AAATTAGTATAGGAATTGAATGTGATAAAATTGAAGGCACAACAGTTGATACCGATCTAG
ch1                  AAATTAGTATAGGAATTGAATGTGATAAAATTGAAGGCACAACAGTTGATACCGATCTAG
                     ************************************************************
```

```
su_mut                    CAGTTCAGTTGAACTTGTTAGTGTATATATTCCACAATTGATTGAGTTATCATTACTTAA
su_Di Genova              CAGTTCAGTTGAACTTGTTAGTGTATATATTCCACAATTGATTGAGTTATCATTACTTAA
ch2                       CAGTTCAGTTGAACTTGTTAGTGTATATATTTAACAATTGATTGAGCTATCATTACTTAA
VIT_218S0041G01880.2      CAGTTCAGTTGAACTTGTTAGTGTATATATTTAACAATTGATTGAGCTATCATTACTTAA
su_wt                     CAGTTCAGTTGAACTTGTTAGTGTATATATTTAACAATTGATTGAGCTATCATTACTTAA
ch1                       CAGTTCAGTTGAACTTGTTAGTGTATATATTTAACAATTGATTGAGCTATCATTACTTAA
                          ****************************   ************ *************


su_mut                    TTTTTTTGATGTATGAAAACTATCCATATCCATACTACTACTCTTCATTGAGCTCTGCTG
su_Di Genova              TTTTTTTGATGTATGAAAACTATCCATATCCATACTACTACTCTTCATTGAGCTCTGCTG
ch2                       TTATTTTGATGTATGAAAACTATCCATATCCATACTACTACTCTTCATTGAGCTCTGCTG
VIT_218S0041G01880.2      TTATTTTGATGTATGAAAACTATCCATATCCATACTACTACTCTTCATTGAGCTCTGCTG
su_wt                     TTATTTTGATGTATGAAAACTATCCATATCCATACTACTACTCTTCATTGAGCTCTGCTG
ch1                       TTATTTTGATGTATGAAAACTATCCATATCCATACTACTACTCTTCATTGAGCTCTGCTG
                          ** *********************************************************


su_mut                    CATTAAACTTTCAGATTGCAGAAGTGGAGAGGCTTCAGCAAGCAAACATGGTGTCAACAC
su_Di Genova              CAYTAAACTTTCAGATTGCAGAAGTGGAGAGGCTTCAGCAAGCAAACATGGTATCAACAC
ch2                       CACTAAACTTTCAGATTGCAGAAGTGGAGAGGCTTCAGCAAGCAAACATGGTATCAACAC
VIT_218S0041G01880.2      CACTAAACTTTCAGATTGCAGAAGTGGAGAGGCTTCAGCAAGCAAACATGGTATCAACAC
su_wt                     CACTAAACTTTCAGATTGCAGAAGTGGAGAGGCTTCAGCAAGCAAACATGGTATCAACAC
ch1                       CACTAAACTTTCAGATTGCAGAAGTGGAGAGGCTTCAGCAAGCAAACATGGTATCAACAC
                          ** ************************************************** *******


su_mut                    ATGAGTTTAATGCCATCCAGGCATTAGTTTCTCTCAATTTCTTTCAGCCCAATATGATTG
su_Di Genova              ATGAGTTCAATGCCATCCAGGCATTAGTTTCTCTCAATTTCTTTCAGCCCAATATGATTG
ch2                       ATGAGTTCAATGCCATCCAGGCATTAGTTTCTCGCAATTTCTTTCAGCCCAATATGATTG
VIT_218S0041G01880.2      ATGAGTTCAATGCCATCCAGGCATTAGTTTCTCGCAATTTCTTTCAGCCCAATATGATTG
su_wt                     ATGAGTTCAATGCCATCCAGGCATTAGTTTCTCGCAATTTCTTTCAGCCCAATATGATTG
ch1                       ATGAGTTCAATGCCATCCAGGCATTAGTTTCTCGCAATTTCTTTCAGCCCAATATGATTG
                          ******* *********************** ************************


su_mut                    AAGGTGGATCCGCAGGCTACCCACTTCCTGATAAGAAGGTCCTCCATCTCGGGTACACTC
su_Di Genova              AGGGTGGATCCACAGGCTACCCACTTCCTGATAAGAAGGTCCTCCATCTCGGGTACACTC
ch2                       AGGGTGGATCCACAGGCTACCCACTTCCTGATAAGAAGGTCCTCCATCTCGGGTACACTC
VIT_218S0041G01880.2      AGGGTGGATCCACAGGCTACCCACTTCCTGATAAGAAGGTCCTCCATCTCGGGTACACTC
su_wt                     AGGGTGGATCCACAGGCTACCCACTTCCTGATAAGAAGGTCCTCCATCTCGGGTACACTC
ch1                       AGGGTGGATCCACAGGCTACCCACTTCCTGATAAGAAGGTCCTCCATCTCGGGTACACTC
                          * ********* ************************************************


su_mut                    ATCTTCCGCCATGTTTATTCATTTGCATTCTTTCTCGGTCTAATATTGAAGATTAAAAGA
su_Di Genova              ATCTTTCGCCATGTTTATTCATTTGCATTCTTTCTCGGTCTAATATTGAAGATTAAAAGA
ch2                       ATCTTTCGCCATGTTTATTCATTTGCATTCTTTCTCGGTCTAATATTGAAGATTAAAAGA
VIT_218S0041G01880.2      ATCTTTCGCCATGTTTATTCATTTGCATTCTTTCTCGGTCTAATATTGAAGATTAAAAGA
su_wt                     ATCTTTCGCCATGTTTATTCATTTGCATTCTTTCTCGGTCTAATATTGAAGATTAAAAGA
ch1                       ATCTTTCGCCATGTTTATTCATTTGCATTCTTTCTCGGTCTAATATTGAAGATTAAAAGA
                          ***** ******************************************************


su_mut                    GCACATAAATCTCCAAGTAGCCTTAGGTCTGCTAAATTCATCACTACAAATGGTATATGC
su_Di Genova              GCACATAAATCWCCAAGTAGCCTTAGGTCTGCTAAATTCATCACTACAAATGGTATATGC
ch2                       GCACATAAATCACCAAGTAGCCTTAGGTCTGCTAAATTCATCACTACAAATGGTATATGC
VIT_218S0041G01880.2      GCACATAAATCACCAAGTAGCCTTAGGTCTGCTAAATTCATCACTACAAATGGTATATGC
su_wt                     GCACATAAATCACCAAGTAGCCTTAGGTCTGCTAAATTCATCACTACAAATGGTATATGC
ch1                       GCACATAAATCACCAAGTAGCCTTAGGTCTGCTAAATTCATCACTACAAATGGTATATGC
                          *********** ************************************************


su_mut                    ATGAATAGCTTAATGAGACATTTCAAGCCATGCATTGGGTAGAAAAAAGTTGTGAGATTT
su_Di Genova              ATGAAYAGCTTAATGAGACATTTCAAGCCATGCATTGGGTAGAAAAAAGTTGTGAGATTT
ch2                       ATGAACAGCTTAATGAGACATTTCAAGCCATGCATTGGGTAGAAAAAAGTTGTGAGATTT
VIT_218S0041G01880.2      ATGAACAGCTTAATGAGACATTTCAAGCCATGCATTGGGTAGAAAAAAGTTGTGAGATTT
su_wt                     ATGAACAGCTTAATGAGACATTTCAAGCCATGCATTGGGTAGAAAAAAGTTGTGAGATTT
ch1                       ATGAACAGCTTAATGAGACATTTCAAGCCATGCATTGGGTAGAAAAAAGTTGTGAGATTT
                          ***** ******************************************************


su_mut                    CTTCGTCTTTTAGAATACTGCATAGTCATTGACTACCCTATATAATCTTGCTGTGAATTA
su_Di Genova              CTTCGTCTTTTARAATACTGCATAGTCATTGACTACCCTATATAATCTTGCTGTGAATTA
ch2                       CTTCGTCTTTTAAAATACTGCATAGTCATTGACTACCCTATATAATCTTGCTGTGAATTA
VIT_218S0041G01880.2      CTTCGTCTTTTAAAATACTGCATAGTCATTGACTACCCTATATAATCTTGCTGTGAATTA
su_wt                     CTTCGTCTTTTAAAATACTGCATAGTCATTGACTACCCTATATAATCTTGCTGTGAATTA
ch1                       CTTCGTCTTTTAAAATACTGCATAGTCATTGACTACCCTATATAATCTTGCTGTGAATTA
```

```
              ***********  *************************************************
su_mut          TATACTAATTATGATGGGCAAAACAAAAATTTGTTCTCCAAATCCTCCTCAAGTTCCTAT
su_Di Genova    TATACTAATTATGATGGGCAAAACAAAAATTTGTTCTCCAAATCCTCCTCAAGTTCCTAT
ch2             TATACTAATTATGATGGGCAAAACAAAAATTTGTTCTCCAAATCCTCCTCAAGTTCCTGT
VIT_218S0041G01880.2 TATACTAATTATGATGGGCAAAACAAAAATTTGTTCTCCAAATCCTCCTCAAGTTCCTGT
su_wt           TATACTAATTATGATGGGCAAAACAAAAATTTGTTCTCCAAATCCTCCTCAAGTTCCTGT
ch1             TATACTAATTATGATGGGCAAAACAAAAATTTGTTCTCCAAATCCTCCTCAAGTTCCTGT
              *********************************************************  *
su_mut          GATGTGTATGACGGCCTAAAGATGATTGTTCATCTGGGCATTTCGATTTTTGCTCATCCT
su_Di Genova    GATGTGTATGACGGCCTAAAGATGATTGTTCATCTGGGCATTTCGATTTTTGCTCATCCT
ch2             GATGTGTATGAGGGCCTAAAGATGATTGTTCATCTGGGCATTTCGATTTTTGCTCATCCT
VIT_218S0041G01880.2 GATGTGTATGAGGGCCTAAAGATGATTGTTCATCTGGGCATTTCGATTTTTGCTCATCCT
su_wt           GATGTGTATGAGGGCCTAAAGATGATTGTTCATCTGGGCATTTCGATTTTTGCTCATCCT
ch1             GATGTGTATGAGGGCCTAAAGATGATTGTTCATCTGGGCATTTCGATTTTTGCTCATCCT
              ***********  ***********************************************


su_mut          TAATAATCAGTACTTCTCCAAAGTAAGTAGATTTCCATGTTTTCTTTTGGCTTACAATAA
su_Di Genova    TAATAATCAGTACTTCTCCAAAGTAAGTAGATTTCCATGTTTTCTTTTGGCTTACAATAA
ch2             TAATAATCAGTACTTCTCCAAAGTAAGTAGATTTCCATGTTTTCTTTTGGCTTACAATAA
VIT_218S0041G01880.2 TAATAATCAGTACTTCTCCAAAGTAAGTAGATTTCCATGTTTTCTTTTGGCTTACAATAA
su_wt           TAATAATCAGTACTTCTCCAAAGTAAGTAGATTTCCATGTTTTCTTTTGGCTTACAATAA
ch1             TAATAATCAGTACTTCTCCAAAGTAAGTAGATTTCCATGTTTTCTTTTGGCTTACAATAA
              ************************************************************


su_mut          GATTCAAGTTCGACCTAATTCCTGGGGTTTTTTGCTTTTTGTTTTATGTAGGTAAATGAT
su_Di Genova    GATTCAAGTTCGACCTAATTCCTGGGGTTTTTTGCTTTTTGTTTTATGTAGGTAAATGAT
ch2             GATTCAAGTTCGACCTAATTCCTGGGGTTTTTTGCTTTTTGTTTTATGTAGGTAAATGAT
VIT_218S0041G01880.2 GATTCAAGTTCGACCTAATTCCTGGGGTTTTTTGCTTTTTGTTTTATGTAGGTAAATGAT
su_wt           GATTCAAGTTCGACCTAATTCCTGGGGTTTTTTGCTTTTTGTTTTATGTAGGTAAATGAT
ch1             GATTCAAGTTCGACCTAATTCCTGGGGTTTTTTGCTTTTTGTTTTATGTAGGTAAATGAT
              ************************************************************
su_mut          GGAGGAAAATATCCGCCAACTTCATCTCCTTATGATGTTTAAATAAATCTTTTCCCATG
su_Di Genova    GGRAGAAAATATCCGCCAACTTCATCTCCTTATGATGTTTAAATAAATCTTTTCCCATG
ch2             GGGAGAAAATATCCGCCAACTTCATCTCCTTATGATGTTTAAATAAATCTTTTCCCATG
VIT_218S0041G01880.2 GGGAGAAAATATCCGCCAACTTCATCTCCTTATGATGTTTAAATAAATCTTTTCCCATG
su_wt           GGGAGAAAATATCCGCCAACTTCATCTCCTTATGATGTTTAAATAAATCTTTTCCCATG
ch1             GGGAGAAAATATCCGCCAACTTCATCTCCTTATGATGTTTAAATAAATCTTTTCCCATG
              **  ********************************************************


su_mut          CTATCCAAACTGTTATGTAACGGAGAATTAATGTCATTGTTGTATTTGGGGTTTCTATGA
su_Di Genova    CTATCCAAACTGTTATGTAACGGAGAATTAATGTCATTGTTGTATTTGGGGTTTCTATGA
ch2             CTATCCAAACTGTTATGTAACGGAGAATTAATGTCATTGTTGTATTTGGGGTTTCTATGA
VIT_218S0041G01880.2 CTATCCAAACTGTTATGTAACGGAGAATTAATGTCATTGTTGTATTTGGGGTTTCTATGA
su_wt           CTATCCAAACTGTTATGTAACGGAGAATTAATGTCATTGTTGTATTTGGGGTTTCTATGA
ch1             CTATCCAAACTGTTATGTAACGGAGAATTAATGTCATTGTTGTATTTGGGGTTTCTATGA
              ************************************************************


su_mut          CTTGGTATTTATCAGCTAGCAACCAGTACTGTTTTGTATAACATTATGACATATAATATA
su_Di Genova    CTTGGTATTTATCMGCTAGCAACCAGTACTGTTTTGTATAACATTATGATATATAATATA
ch2             CTTGATATTTATCCGCTAGCAACCAGTACTGTTTTGTATAACATTATGATATATAATATA
VIT_218S0041G01880.2 CTTGGTATTTATCCGCTAGCAACCAGTACTGTTTTGTATAACATTATGATATATAATATA
su_wt           CTTGGTATTTATCCGCTAGCAACCAGTACTGTTTTGTATAACATTATGATATATAATATA
ch1             CTTGGTATTTATCCGCTAGCAACCAGTACTGTTTTGTATAACATTATGATATATAATATA
              ****  ******** ******************************** **********


su_mut          GCCTATTAAAAT
su_Di Genova    GCCTATTAAAAT
ch2             GCCTATTAAAAT
VIT_218S0041G01880.2 GCCTATTAAAATATACAATATTCCTAATCT
su_wt           GCCTATTAAAAT
ch1             GCCTATTAAAATATACAATATTCCTAATC
              ************************
```

Supplementary Fig. 1. Complete alignment of *VvAGL11* alleles. The sequences presented in the alignment are *VvAGL11* PN40024 (VIT_218S0041G01880.2), *VvAGL11*

'Chardonnay allele 1' (CH1), *VvAGL11* 'Chardonnay allele 2' (CH2), *VvAGL11* 'Sultanina' mutant allele (SU *mut*), *VvAGL11* 'Sultanina wt allele' (SU *wt*) and the available sequence from 'Sultanina' by Di Genova (2014). Underlined sequence represents the SSR marker VMC7F2, blue sequence marks represent UTRs, red sequences comprise exons and green enhancement are indicating putative CArG-Box sequences.
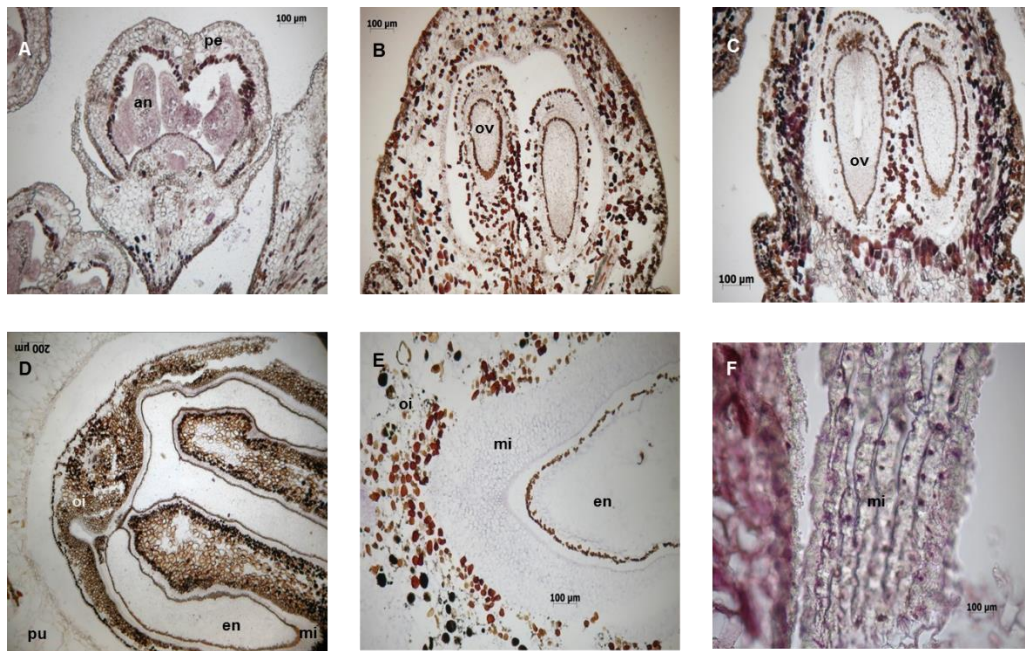
Supplementary Fig. 2. *In situ* hybridization of *VvAGL11* sense probe (control) in 'Chardonnay'. H1 flower stage **(A)**, pre anthesis flower stage **(B)**, fruit set flower stage **(C)**, 2 weeks **(D)**, 4 weeks **(E)** and 6 weeks **(F)** of fruit development. Anther (an), endosperm (en), medium integument (mi), outer integument (oi), ovule (ov), petal (pe), pulp (pu).
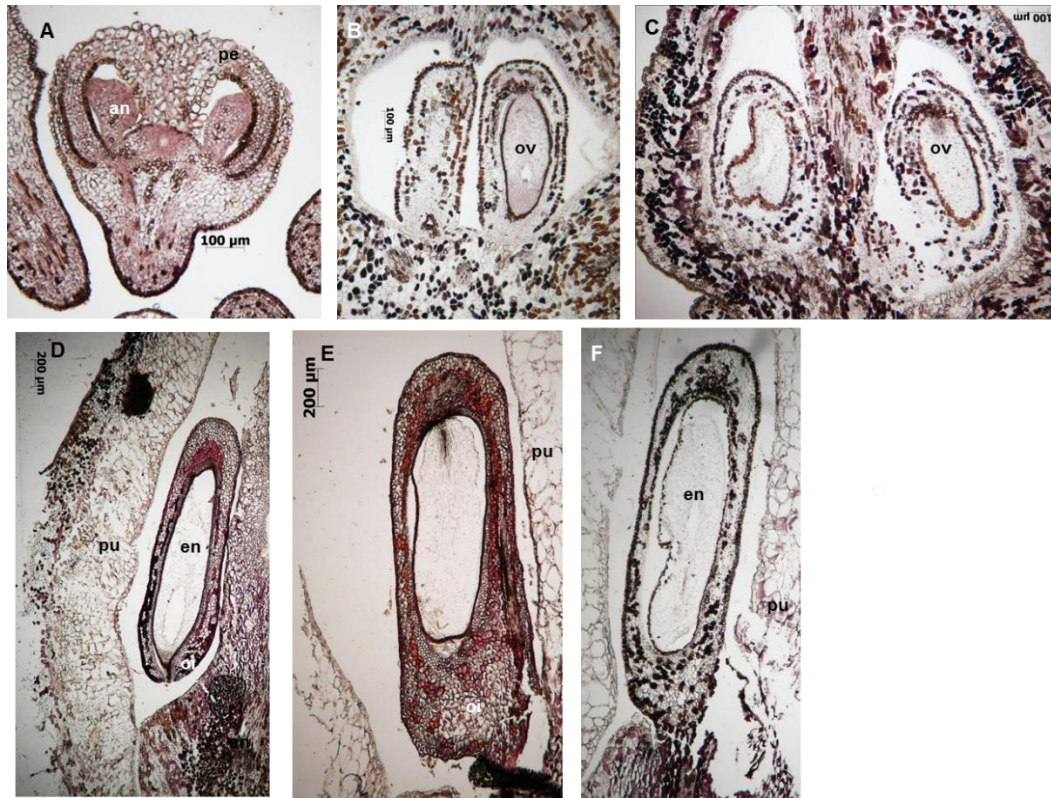
Supplementary Fig. 3. *In situ* hybridization of *VvAGL11* sense probe (control) in 'Sultanina'. H1 flower stage **(A)**, pre anthesis flower stage **(B)**, fruit set flower stage **(C)**, 2 weeks **(D)**, 4 weeks **(E)** and 6 weeks **(F)** of fruit development. Anther (an), endosperm (en), medium integument (mi), outer integument (oi), ovule (ov), petal (pe), pulp (pu).

4 CONCLUSÕES

A utilização da técnica de sequenciamento alelo-específica mostrou-se eficiente na identificação das variações alélicas de *VvAGL11* em 'Chardonnay' e 'Sultanina'. Foram identificados conjuntos de *SNPs* e *INDELs* que permitem diferenciar os alelos avaliados. Com isso, foi possível discernir dois alelos para 'Chardonnay' e dois para 'Sultanina'. Um dos alelos de 'Sultanina', o alelo SU *mut*, é o único que possui mutações pontuais em seu RNA mensageiro, estas compostas por 10 *SNPs*, dois deles realizando modificações de aminoácidos na região codificadora. O sequenciamento por *RACE* permitiu a identificação dos *mRNAs* de *VvAGL11* de cada cultivar, mostrando que os quatro alelos são transcritos, porém 'Chardonnay' possui um alelo, CH1, com uma região codificante 60 nucleotídeos maior do que os outros alelos avaliados.

A quantificação de transcritos por meio da técnica de *RT-qPCR* permitiu a avaliação das isoformas de transcritos de *VvAGL11* previamente identificadas por meio de *RACE*. A expressão gênica relativa de cada isoforma foi avaliada em amostras de ambas as cultivares nos estádios fenológicos de flores e frutos. As isoformas de *VvAGL11* identificadas em 'Sultanina' são igualmente pouco expressas em todos os estádios analisados, enquanto que as isoformas de 'Chardonnay' também são pouco expressas em flores e polpa. Em contraste, os alelos de CH1 e CH2 possuem uma expressão 15-65 vezes maior em sementes em comparação com flores e frutos tanto de 'Chardonnay' como de 'Sultanina'.

Para a investigação de diferenças morfológicas entre as sementes de 'Chardonnay' e o traço de semente de 'Sultanina', o emblocamento dos tecidos em resina plástica foi realizado. Isto permitiu a observação da provável perda de identidade da camada de integumento médio do traço de semente, o qual não se alongou nem se duplicou como

esperado em quatro semanas, mantendo sua forma original indiferenciada durante todo o desenvolvimento do fruto.

A caracterização da expressão tecido-específica de *VvAGL11* foi realizada com sucesso por meio da investigação do padrão de expressão espaço temporal do gene pela técnica de hibridização *in situ*. Ambas as cultivares e os estádios de desenvolvimento de flor e fruto utilizadas nos ensaios de *RT-qPCR* foram analisados. O acúmulo dos transcritos de *VvAGL11* foi detectado especificamente na camada de integumento médio da semente de 'Chardonnay', a qual é responsável pelo alongamento da casca em quatro semanas. Esta é justamente a camada que apresentou atraso de desenvolvimento na cultivar estenoespermocárpica Sultanina. Todavia, não foi detectada hibridização do gene na cultivar apirênica 'Sultanina', nem mesmo no traço de semente analisado. Os resultados são consistentes com a hipótese que a expressão de *VvAGL11* é necessária para a formação da semente e a análise dos dados permite sugerir que a diminuição de sua expressão pode estar relacionada à perda de identidade da camada de integumento médio da semente o que afeta seu tamanho final e leva ao fenótipo de apirenia.

5 PERSPECTIVAS

* Caracterizar a funcionalidade de *VvAGL11* por meio de complementação em modelo heterólogo (*A. thaliana*);

* Avaliar a ortologia de *VvAGL11* e *AtAGL11* por meio da complementação de mutante *stk* (*seedstick*);

* Caracterizar funcionalmente *VvAGL11* em videiras por meio da expressão do gene em cultivares apirênicas e do seu silenciamento em cultivares pirênicas;

* Caracterizar as interações da proteína VvAGL11 com seus genes-alvo e os elementos *cis* (CArG-box) críticos às interações;

* Identificar as proteínas que constituem complexos quaternários de transcrição juntamente com VvAGL11;

* Identificar um haplótipo associado com potencial para seleção assistida da apirenia em videira;

* Desenvolver e testar um marcador definitivo para apirenia a partir de SNPs do alelo *VvAGL11* de Sultanina mutado, almejando a geração de patente sobre essas marcas;

* Avaliar cinco populações segregantes para o fenótipo de apirenia (1.000 indivíduos) com os haplótipos de marcadores microssatélites e com o marcador definitivo.

6 REFERÊNCIAS BIBLIOGRÁFICAS

ADAM-BLONDON, A. F.; LAHOGUE-ESNAULT, F.; BOUQUET, A.; BOURSIQUOT, J. M.; THIS, P. Usefulness of two SCAR markers for marker-assisted selection of seedless grapevine cultivars. *Vitis*, 40: 147–155. 2001.

ADAM-BLONDON, A. F.; ROUX, C.; CLAUX, D.; BUTTERLIN, G.; MERDINOGLU, D.; THIS, P. Mapping 245 SSR markers on the *Vitis vinifera* genome: a tool for grape genetics. *Theoretical and Applied Genetics*, 109 (5): 1017-1027. 2004.

ADAMCZYK, B. J. & FERNANDEZ, D. E. *MIKC** MADS-domain heterodimers are required for pollen maturation and tube growth in *Arabidopsis*. *Plant Physiology,* 149: 1713–1723. 2009.

AMARAL, A. L.; CAMARGO, U. A.; OLIVEIRA, P. R. D. Uva sem sementes: uso da biotecnologia na busca de novas cultivares apirênicas. *Biotecnologia Ciência e Desenvolvimento*, 2 (10): 108-112. 1999.

ANGENENT, G. C.; COLOMBO, L. Molecular control of ovule development. *Trends in Plant Science*, 1 (7): 228–232. 1996.

ANGENENT, G. C.; FRANKEN, J.; BUSSCHER, M.; VAN DIJKEN, A.; VAN WENT, J. L.; DONS, H. J. M.; VAN TUNEN, A. J. A novel class of MADS-box genes is involved in ovule development in petunia. *The Plant Cell*, 7: 1569–1582. 1995.

ANONYMOUS. Descriptor list for grapevine varieties and *Vitis* species 243. *Organization Internacional de la Vigne et du Vin* (OIV), Paris. 1983.

BERGER, F.; GRINI, P. E.; SCHNITTGER, A. Endosperm: an integrator of seed growth and development. *Current Opinion in Plant Biology*, 9: 664–670. 2006.

BOSS, P. K.; SENSI, E.; HUA, C.; DAVIES, C.; THOMAS, M. R. Cloning and characterization of grapevine (*Vitis vinifera* L.) MADS-box genes expressed during inflorescence and berry development. *Plant Science,* 162: 887–895. 2002.

BOSS, P. K.; VIVIER, M.; MATSUMOTO, S.; DRY, I. B.; THOMAS, M. R. A cDNA from grapevine (*Vitis vinifera* L.), which shows homology to AGAMOUS and SHATTERPROOF, is not only expressed in flowers but also throughout berry development. *Plant Molecular Biology,* 45, 541–553. 2001.

BOUQUET, A. & DANGLOT, Y. Inheritance of seedlessness in grapevine (*Vitis vinifera* L.). *Vitis,* 35: 35-42. 1996.

BRAMBILLA, V.; BATTAGLIA, R.; COLOMBO, M.; MASIERO, S.; BENCIVENGA, S.; KATER, M.; COLOMBO, L. Genetic and molecular interactions between BELL1 and

MADS box factors support ovule development in *Arabidopsis. The Plant Cell,* 19 (8): 2544–2556. 2007.

CABEZAS, J. A.; CERVERA, M. T.; RUIZ-GARCIA, L.; CARRENO, J.; MARTINEZ-ZAPATER, J. M. A genetic analysis of seed and berry weight in grapevine. *Genome,* 49 (12):1572–1585, 2006.

CAMARGO, U. A. Uvas sem sementes cultivares BRS Morena, BRS Clara e BRS Linda. (Sistema de Produção, 8 ISSN 1678-8761, Versão Eletrônica). Disponível em: <http://sistemasdeproducao.cnptia.embrapa.br/FontesHTML/Uva/UvasSemSementes/culti vares.htm>. 2005.

CHRISTENSEN, L. P. Raisin Grape Varieties. Pages 38-47 in: *Raisin Production Manual*. University of California, Agricultural and Natural Resources Publication 3393, Oakland, CA. 2000.

COLOMBO, L.; FRANKEN, J.; KOETJE, E.; VAN WENT, J.; DONS, H. J.; ANGENENT, G. C.; VAN TUNEN, A. J. The petunia MADS box gene FBP11 determines ovule identity. *The Plant Cell,* 7 (11):1859-1868. 1995.

COLOMBO, L.; FRANKEN, J.; VANDERKROL, A. R.; WITTICHY, P. E.; DONSY, H. J. M.; ANGENENT, G. C. Downregulation of ovule-specific MADS box genes from petunia results in maternally controlled defects in seed development. *The Plant Cell,* 9: 703–715, 1997.

COLOMBO, M.; BRAMBILLA, V.; MARCHESELLI, R.; CAPORALI, E.; KATER, M. M.; COLOMBO, L. A new role for the SHATTERPROOF genes during *Arabidopsis* gynoecium development. *Development Biology*, 337: 294-302. 2010.

COSTANTINI, L.; BATTILANA, J.; LAMAJ, F.; FANIZZA, G.; GRANDO, M.S. Berry and phenology-related traits in grapevine (*Vitis vinifera* L.): from quantitative trait loci to underlying genes. *BioMedCentral Plant Biology*, 8 (38): 1471-2229. 2008.

DALBÒ, M. A.; YE, G. N.; WEEDEN, N. F.; STEINKELLNER, H.; SEFC, K. M.; REISCH, B. I. A gene controlling sex in grapevines placed on a molecular marker-based genetic map. *Genome,* 43: 333-340. 2000.

DE BODT, S.; RAES, J.; FLORQUIN, K.; ROMBAUTS, S.; ROUZÉ, P.; THEISSEN, G.; VAN DE PEER, Y. Genome wide structural annotation and evolutionary analysis of the type I MADS-box genes in plants. *Journal of Molecular Evolution,* 56: 573-586. 2003.

DEYHOLOS, M. K. & SIEBURTH, L. E. Separable whorl-specific expression and negative regulation by enhancer elements within the *AGAMOUS* second intron. *The Plant Cell,* 12: 1799–1810. 2000.

DI GENOVA, A.; ALMEIDA, A. M.; MUÑOZ-ESPINOZA, C.; VIZOSO, P.; TRAVISANY, D.; MORAGA, C.; PINTO, M.; HINRICHSEN, P.; ORELLANA, A.; MAASS, A. Whole genome comparison between table and wine grapes reveals a comprehensive catalog of structural variants. *BMC Plant Biology*, 7 (14): 1471-2229. 2014.

DOLIGEZ, A.; BOUQUET, A.; DANGLOT, Y.; LAHOGUE, F.; RIAZ, S.; MEREDITH, C. P.; EDWARDS, K. J.; THIS, P. Genetic mapping of grapevine (*Vitis vinifera* L.) applied to the detection of QTLs for seedlessness and berry weight. *Theoretical and Applied Genetics,* 105 (5):780–795, 2002.

DORNELAS, M. C. & DORNELAS, O. From leaf to flower: revisiting Goethe's concepts on the ¨metamorphosis¨ of plants. *Brazilian Journal of Plant Physiology*, 17 (4): 335-343. 2005.

EGLI, D. B. In: Seed biology and the yield of grain crops. *CAB International*. ISBN 0852992412. 178 p. 1998.

EMERSHAD, R. L.; RAMMING, D. W.; SERPE, M. D. In: Ovulo embryo development and plant formation from stenospermic genotypes of *Vitis vinifera*. *American Journal of Botany*. 1989.

FANIZZA, G.; LAMAJ, F.; COSTANTINI, L.; CHAABANE, R.; GRANDO, M. S. QTL analysis for fruit yield components in table grapes (*Vitis vinifera*). *Theoretical and Applied Genetics,* 111: 658–664. 2005.

FAO, Food and Agriculture Organization of the United Nations. Disponível em <http://www.faostats.org/>. Acesso em 22 de junho de 2014.

FASOLI, M.; DAL SANTO, S.; ZENONI, S. The grapevine expression atlas reveals a deep transcriptome shift driving the entire plant into a maturation program. *The Plant Cell,* 24: 3489-3505. 2012.

FAVARO, R.; PINYOPICH, A.; BATTAGLIA, R.; KOOIKER, M.; BORGHI, L.; DITTA, G.; YANOFSKY, M. F.; KATER, M.; COLOMBO, L. MADS-Box protein complexes control carpel and ovule development in *Arabidopsis*. *The Plant Cell,* 15: 2603-2611. 2003.

FISCHER, B. M.; SALAKHUTDINOV, I.; AKKURT, M.; EIBACH, R.; EDWARDS, K. J.; TÖPFER, R.; ZYPRIAN, E. M. Quantitative trait locus analysis of fungal disease resistance factors on a molecular map of grapevine. *Theoretical and Applied Genetics*, 108: 501-515. 2004.

GARCIA, D.; FITZ-GERALD, J. N.; BERGER, F. Maternal control of integument cell elongation and zygotic control of endosperm growth are coordinated to determine seed size in *Arabidopsis*. *The Plant Cell,* 17: 52–60. 2005.

GARCIA, D.; SAINGERY, V.; CHAMBRIER, P.; MAYER, U.; JURGENS, G.; BERGER, F. *Arabidopsis* haiku mutants reveal new controls of seed size by endosperm. *Plant Physiology*, 131: 1661-1670. 2003.

GRATTAPAGLIA, D. & SEDEROFF, R. Genetic linkage maps of Eucalyptus grandis and Eucalyptus urophylla using a pseudotestcross: mapping strategy and RAPD markers. *Genetics*, 137: 1121–1137. 1994.

HAUGHN, G. & CHAUDHURY, A. Genetic analysis of seed coat development in *Arabidopsis*. *Trends Plant Science*, 10: 472-477. 2005.

HONG, R. L.; HAMAGUCHI, L.; BUSCH, M. A.; WEIGEL, D. Regulatory elements of the floral homeotic gene AGAMOUS identified by phylogenetic footprinting and shadowing. *The Plant Cell*, 16: 1296-1309. 2003.

HUANG, K.; LOUIS, J. M.; DONALDSON, L.; LIM, F. L.; SHARROCKS, A. D.; CLORE, G. M. Solution structure of the MEF2A-DNA complex: structural basis for the modulation of DNA bending and specificity by MADS-box transcription factors. *EMBO Journal,* 19: 2615-2628. 2000.

JAILLON, O.; AURY, J; M.; NOEL, B. The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. *Nature,* 449: 463–467. 2007.

JURGENS, G. & MAYER, U. *Arabidopsis*. In: Embryos: Colour Atlas of Development, J. B. L. Bard, ed (*London: Wolfe Publishing*): 7–21. 1994.

KAUFMANN, K.; MELZER, R.; THEISSEN, G. *MIKC*-type MADS-domain proteins: structural modularity, protein interactions and network evolution in land plants. *Gene,* 347: 183-198. 2005.

KOOIKER, M.; AIROLDI, C. A.; LOSA, A.; MANZOTTI P. S.; FINZI, L.; KATER M. M.; COLOMBO, L. BASIC PENTACYSTEINE 1, a GA binding protein that induces conformational changes in the regulatory region of the homeotic *Arabidopsis* gene SEEDSTICK. *The Plant Cell,* 17: 722–729. 2005.

KRAMER, E. M.; JARAMILLO, M. A.; DI STILIO, V. S. Patterns of gene duplication and functional evolution during the diversification of the AGAMOUS subfamily of MADS box genes in angiosperms. *Genetics*, 166 (2): 1011-1023. 2004.

LACIRIGNOLA, C. & DIGIARO, M. Grapevine industry in the world: state of the art and future trends. In: Proceedings of the Mediterranean network on grapevine clostero viruses. *Ciheam,* 33-46. 1999.

LAHOGUE, F.; THIS, P.; BOUQUET, A. Identification of a codominant marker linked to the seedlessness character in grapevine. *Theoretical and Applied Genetics,* 97: 1638-1649. 1998.

LAZZAROTTO, J.J.; FIORAVANÇO, J. C. Comércio exterior mundial e brasileiro de uva de mesa: análise de indicadores de competitividade, tendência e sazonalidades. *Documentos Embrapa Uva e Vinho*, 44-54. 2012.

LIU,C.; CHEN, H.; ER, H. L.; SOO, H. M.; KUMAR, P. P.; HAN, J. H.; LIOU, Y. C.; YU, H. Direct interaction of AGL24 and SOC1 integrates flowering signals in *Arabidopsis*. *Development,* 135: 1481-1491. 2008.

LODHI, M. A.; DALY, M. J. Y.; WEEDEN, N. F.; REISCH, B. I. A molecular marker based linkage map of *Vitis*. *Genome*, 38: 786–794. 1995.

LOHMANN, J. U. & WEIGEL, D. Building beauty: the genetic control of floral patterning. *Development Cell*, 2: 135–142. 2002.

LOOMIS, N. H. & WEINBERG, J. H. Inheritance studies of seedlessness in grape. *Journal of American Society of Horticulture Science,* 104: 181-184. 1979.

MATIAS-HERNANDEZ, L.; BATTAGLIA, R.; GALBIATI, F.; RUBES, M.; EICHENBERGER, C.; GROSSNIKLAUS, U.; KATER, M. M.; COLOMBO, L. VERDANDI is a direct target of the MADS domain ovule identity complex and affects embryo sac differentiation in *Arabidopsis*. *The Plant Cell,* 22: 1702–1715. 2010.

MEJÍA, N.; GEBAUER, M.; MUNOZ, L.; HEWSTONE, N.; MUNOZ, C.; HINRICHSEN, P. Identification of QTLs for seedlessness, berry size, and ripening date in a seedless x seedless table grape progeny. *American Journal of Enology and Viticulture,* 58 (4): 499–507. 2007.

MEJÍA, N.; SOTO, B.; GUERRERO, G.; CASANUEVA, X.; HOUEL, C.; MICCONO, M. L. A.; RAMOS, R.; LE CUNFF, L.; BOURSIQUO, J. M.; HINRICHSEN, P. ADAM-BLONDO, A. F. Molecular, genetic and transcriptional evidence for a role of *VvAGL11* in stenospermocarpic seedlessness in grapevine. *BMC Plant Biology*, 11 (57): 1471-2229. 2011.

MELZER, R. & THEISSEN, G. Reconstitution of 'floral quartets' in vitro involving class B and class E floral homeotic proteins. *Nucleic Acids Research,* 37: 2723–2736. 2009.

MENDES, M. A.; GUERRA, R. F.; BERNS, M. C.; MANZO, C.; MASIERO, S.; FINZI, L. MADS domain transcription factor mediate short-range looping that is essential for target gene expression in *Arabidopsis*. *The Plant Cell,* 25 (7): 2560-2572. 2013.

NORMAN, C.; RUNSWICK, M.; POLLOCK, R.; TREISMAN, R. Isolation and properties of cDNA clones encoding SRF, a transcription factor that binds to the c-fosserum response element. *Cell,* 55 (6): 989-1003. 1988.

OIV. Organization Internacional de la Vigne et du Vin (OIV), Paris. Statiques du secteur vitivinicole mondial. Disponível em <http://www.oiv.int/oiv/cms/index>. Acesso em 26 de junho de 2014.

PARENICOVÁ, L.; DE FOLTER, S.; KIEFFER, M.; HORNER, D. S.; FAVALLI, C.; BUSSCHER, J.; COOK, H. E.; INGRAM, R. M.; KATER, M. M.; DAVIES, B. Molecular and phylogenetic analyses of the complete MADS-box transcription factor family in *Arabidopsis*: new openings to the MADS world. *The Plant Cell*, 15 (7): 1538-1551. 2003.

PASSMORE, S.; MAINE, G. T.; ELBLE, R.; CHRIST, C.; TYE, B. K. Saccharomyces cerevisiae protein involved in plasmid maintenance is necessary for mating of MAT alpha cells. Journal of *Molecular Biology,* 204: 593-606. 1988.

PELLEGRINI, L.; TAN, S.; RICHMOND, T. J. Structure of serum response factor core bound to DNA. *Nature,* 376: 490-498. 1995.

PINYOPICH, A.; DITTA, G., SAVIDGE, B.; LILJEGREN, SJ.; BAUMANN, E.; WISMAN, E.; YANOFSKY, M. Assessing the redundancy of MADS-box genes during carpel and ovule development. *Nature,* 424: 85–88. 2003.

PRATT, C. Reproductive anatomy in cultivated grapes. A review. *American Journal of Enology and Viticulture,* 22 (2): 92-109. 1971.

PROTAS, J. F. S. Uvas sem sementes cultivares BRS Morena, BRS Clara e BRS Linda. (Sistema de Produção, 8 ISSN 1678-8761, Versão Eletrônica). Disponível em: <http://sistemasdeproducao.cnptia.embrapa.br/FontesHTML/Uva/UvasSemSementes/apres entacao.htm>. *Embrapa Uva e Vinho*. 2005.

REVERS, L. F.; WELTER, L. J.; IRALA, P. B.; SILVA, D. C.; LAMPE, V. S.; OLIVEIRA, P. R. D.; GARRIDO, L. R. Co-Localization of QTLs for seedlessness and downy mildew resistance in grapevine. In: *International Conference on Grapevine Breeding and Genetics.* v. 10: 449-456. 2010.

RIAZ, S.; DANGL, G. S.; EDWARDS, K. J.; MEREDITH, C. P. A microsatellite marker based framework linkage map of *Vitis vinifera* L. *Theoretical and Applied Genetics,* 108: 864–872. 2003.

ROUNSLEY, S. D.; DITTA, G. S.; YANOFSKY M. F. Diverse roles for MADS box genes in *Arabidopsis* development. *The Plant Cell,* 7: 1259–1269. 1995.

SCHMIDT, R. J.; VEIT, B.; MANDEL, M. A.; MENA, M.; HAKE, S.; YANOFSKY, M . F. Identification and molecular characterization of ZAG1 the maize homolog of the *Arabidopsis* floral homeotic gene AGAMOUS. *The Plant Cell,* 5 (7): 729–737. 1993.

SCHWARZ-SOMMER, Z.; HUIJSER, P.; NACKEN, W.; SAEDLER, H.; SOMMER, H. Genetic control of flower development by homeotic genes in *Antirrhinum majus. Science,* 250: 931–936. 1990.

SMACZNIAK, C.; IMMINK, R. G. H.; ANGENENT, G. C.; KAUFMANN, K. Developmental and evolutionary diversity of plant MADS-domain factors: Insights from recent studies. *Development,* 139: 3081–3098. 2012.

SOMMER, H.; BELTRÁN, J. P.; HUIJSER, P.; PAPE, H.; LÖNNIG, W. E.; SAEDLER, H.; SCHWARZ-SOMMER, Z. Deficiens, a homeotic gene involved in the control of flower morphogenesis in Antirrhinum majus: the protein shows homology to transcription factors. *EMBO Journal*, 9 (3): 605–613. 1990.

SONG, I. J.; NAKAMURA, T.; FUKUDA, T.; YOKOYAMA, J.; ITO, T.; ICHIKAWA, H. Spatiotemporal expression of duplicate agamous orthologues during floral development in Phalaenopsis. *Development Genes and Evolution,* 216: 301–313. 2006.

STADLER, R.; LAUTERBACH, C.; SAUER, N. Cell-to-cell movement of green fluorescent protein reveals post-phloem transport in the outer integument and identifies symplastic domains in *Arabidopsis* seeds and embryos. *Plant Physiology,* 139: 701–712. 2005.

STOUT, A. B. Seedlessness in grapes. N.Y. *State Agric. Expt. Stat. Tech. Bull*. 238. 1936.

THEISSEN, G. & SAEDLER, H. Plant biology. Floral quartets. *Nature,* 409: 469-471. 2001.

THEISSEN, G. Development of floral organ identity: stories from the MADS house. *Current Opinion in Plant Biology,* 4: 75-85. 2001.

THEISSEN, G.; SAEDLER, H. MADS-box genes in plant ontogeny and phylogeny. Haeckel's biogenetic law revisited. *Current Opinion in Genetic Development*, 5 (5): 628–639. 1995.

TZENG, T. Y.; CHEN, H. Y.; YANG, C. H. Ectopic expression of carpel-specific MADS box genes from lily and lisianthus causes similar homeotic conversion of sepal and petal in *Arabidopsis*. *Plant Physiology,* 130: 1827–1836. 2002.

UNGRU, A. H. Analysis of communication pathways during seed development in *Arabidopsis thaliana*. Dissertação de mestrado. 2010.

VAROQUAUX, F.; BLANVILLAIN, R.; DELSENY, M.; GALLOIS, P. Less is better: new approaches for seedless fruit production. *Trends in Biotechnology,* 18: 233–242. 2000.

VELASCO, R.; ZHARKIKH, A.; TROGGIO, M. A high quality draft consensus sequence of the genome of a heterozygous grapevine variety. *PLoS ONE,* 2: 1326. 2007.

VITTI, A. Análise da competitividade das exportações brasileiras de frutas selecionadas no mercado internacional. Universidade de São Paulo. Escola superior de agricultura Luis de Queiroz. Dissertação de mestrado. 2009.

WUEST, S. E.; VIJVERBERG, K.; SCHMIDT, A.; WEISS, M.; GHEYSELINCK, J.; LOHR, M.; WELLMER, F.; RAHNENFÜHRER, J.; VON MERING, C.; GROSSNIKLAUS, U. *Arabidopsis* female gametophyte gene expression map reveals similarities between plant and animal gametes. *Current Biology,* 20: 506-512. 2010.

YANOFSKY, M. F. HONG, M. A.; BOWMAN, J. L.; DREWS, G. N.; FELDMANN, K. A.; MEYEROWITZ, E. M. The protein encoded by the *Arabidopsis* homeotic gene AGAMOUS resembles transcription factors. *Nature* 346: 35–39. 1990.

YUN, P. Y.; KIM, S. Y.; OCHIAI, T.; FUKUDA, T.; ITO, T.; KANNO, A. AVAG2 is a putative D-class gene from an ornamental asparagus. *Sexual Plant Reproduction,* 17: 107–116. 2004.

7 CURRICULUM VITAE

DADOS PESSOAIS

Nome: Jaiana Malabarba

Local e data de nascimento: Carlos Barbosa, RS, Brasil. 08/01/1991

E-mail: jaianamalabarba@gmail.com

FORMAÇÃO

2013 – 2014

> **Mestrado em Biologia Celular e Molecular**, PPGBCM, Universidade Federal do Rio Grande do Sul, UFRGS, Brasil. Estrutura do gene *VvAGl11* e análise da expressão durante a morfogênese da semente de videira. Orientador: Dr. Giancarlo Pasquali.

2009 – 2013

> **Graduação em Ciências Biológicas**. Universidade do Vale do Rio dos Sinos, UNISINOS, São Leopoldo, RS, Brasil. Autoecologia De *Aulacoseira Granulata* Ehrenberg (Bacillariophyta) Em Ambientes Lóticos Subtropicais Com Diferentes Graus De Preservação. Drª Luciane Oliveira Crossetti.

FORMAÇÃO COMPLEMENTAR

2011 – 2013

> 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: "Uvas do Brasil: Programa de Melhoramento Genético".

2010 – 2012

Bolsista de Iniciação Científica Petrobras no **Laboratório de Ecofisiologia e Cultura Vegetal**. UNISINOS, São Leopoldo/RS.

Orientação: Drª. Luciane Oliveira Crossetti.

Atuando nos seguintes temas: Estudo das comunidades fitoplânctonicas de diferentes arroios da Bacia do Rio dos Sinos através da avaliação da influência da composição de mata ciliar.

2010 – 2011

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

Orientação: Dr. Celito Crivellaro Guerra.

Atuando nos seguintes temas: Acompanhamento e análises da maturação fenólica, com determinação de taninos e antocianinas presentes em cascas e sementes de diversas variedades de uva provenientes de regiões produtoras dos estados do Rio Grande do Sul e de Santa Catarina no projeto "Novas Regiões Vitivinícolas".

PRÊMIOS E TÍTULOS

2013

1º lugar da Pós-graduação com o trabalho 'Padrão de expressão espaço-temporal do gene *VvAGL11* em tecidos reprodutivos de videira por hibridização *in situ*', Embrapa Uva e Vinho.

2013

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

2012

Laureada como Jovem Cientista em Fruticultura Brasileira, Sociedade Brasileira de Fruticultura.

2012

Distinção em Trabalho de Conclusão de Curso, Universidade do Vale do Rio dos Sinos.

2012

Menção honrosa com o trabalho "Avaliação De Marcadores SSR Com Potencial Para Seleção Assistida De Videiras Apirênicas e Resistentes Ao Míldio" no 2º Prêmio Jovem Cientista em Fruticultura Brasileira, Sociedade Brasileira de Fruticultura.

2011

Destaque em Mostra de Iniciação Científica, Universidade do Vale do Rio dos Sinos.

RESUMOS PUBLICADOS EM ANAIS DE CONGRESSOS

* MALABARBA, J.; BUFFON, V.; PASQUALI, G.; REVERS, L. F. Estudo estrutural e funcional do gene *VvAGL11* e seu papel na morfogênese de sementes de *Vitis vinifera. In:* 12º Encontro de Iniciação Científica e 8º Encontro de Pós-Graduandos da Embrapa Uva e Vinho, Bento Gonçalves. 2014.

* MALABARBA, J.; BUFFON, V.; WAIRICH, A.; MARIATH, J.; GAETA, M.; DORNELAS, M. C.; REVERS, L. F.; PASQUALI, G. Padrão de Expressão Espaço-temporal do Gene *VvAGL11* em Tecidos Reprodutivos de Videira por Hibridização *In situ. In:* X Reunião Anual do Programa de Pós-Graduação em Biologia Celular e Molecular. Porto Alegre: UFRGS, 2013.

* MALABARBA, J.; BUFFON, V.; WAIRICH, A.; MARIATH, J.; GAETA, M.; DORNELAS, M. C.; REVERS, L. F. Gene expression analysis of *VvAGL11* in grapevine reproductive tissues by *In situ* hybridization. *In:* IV Simpósio Brasileiro de Genética Molecular de Plantas, Bento Gonçalves, RS. 2013.

* WAIRICH, A.; MALABARBA, J.; BUFFON, V.; REVERS, L. F. Evaluation of resistance to *Plasmopara viticola* in a self-fertilized segregating population from Villard Blanc (*Vitis* spp.). *In:* IV Simpósio Brasileiro de Genética Molecular de Plantas, Bento Gonçalves, RS. 2013.

∗ MALABARBA, J.; BUFFON, V.; WAIRICH, A.; MARIATH, J.; GAETA, M.; DORNELAS, M. C.; PASQUALI, G.; REVERS, L. F. Padrão de expressão espaço-temporal do gene *VvAGL11* em tecidos reprodutivos de videira por hibridização *In situ*. *In:* 11º Encontro de Iniciação Científica e 7º Encontro de Pós-Graduandos da Embrapa Uva e Vinho, Bento Gonçalves. 2013.

∗ WAIRICH, A.; MALABARBA, J.; BUFFON, V.; REVERS, L. F.; WELTER, L. J. Avaliação da resistência ao *Plasmopara viticola* em uma população segregante para a resistência auto-fertilizada de 'Villard Blanc' (*Vitis* spp.). *In:* 11º Encontro de Iniciação Científica e 5º Encontro de Pós-Graduandos da Embrapa Uva e Vinho, 2012, Bento Gonçalves. 2013.

∗ MALABARBA, J.; WAIRICH, A.; BUFFON, V.; CZERMAINSKI, A.; REVERS, L. F. Avaliação de marcadores SSR com potencial para seleção assistida de videiras apirênicas e resistentes ao míldio. *In:* XXII Congresso Brasileiro de Fruticultura, 2012, Bento Gonçalves. XXII CBF, 2012.

∗ WAIRICH, A.; MALABARBA, J.; BUFFON, V.; CZERMAINSKI, A.; REVERS, L. F. Análise do perfil transcricional de genes candidatos associados à resistência ao míldio em videira, presentes no locus Rpv3. *In:* XXII Congresso Brasileiro de Fruticultura, 2012, Bento Gonçalves. XXII CBF, 2012.

∗ WAIRICH, A.; MALABARBA, J.; BUFFON, V. ; PORTO, D. D. ; REVERS, L. F. Análise do perfil transcricional de genes candidatos associados à resistência ao míldio em videira, presentes no locus Rpv3. *In:* 10º Encontro de Iniciação Científica e 6º Encontro de Pós-Graduandos da Embrapa Uva e Vinho, Bento Gonçalves. 2012.

∗ MALABARBA, J.; WAIRICH, A.; BUFFON, V.; CZERMAINSKI, A.; REVERS, L. F. Avaliação de marcadores SSR com potencial para seleção assistida de videiras apirênicas e resistentes ao míldio. *In:* 10º Encontro de Iniciação Científica e 6º

Encontro de Pós-Graduandos da Embrapa Uva e Vinho, 2012, Bento Gonçalves. 2012.

* MALABARBA, J.; BUFFON, V.; CZERMAINSKI, A.; REVERS, L. F. Caracterização do perfil transcricional de um gene associado à estenoespermocarpia em videira (*Vitis vinifera* L.). III Simpósio de Biodiversidade, UFSM, Santa Maria, RS. 2011.

* MALABARBA, J.; BUFFON, V.; CZERMAINSKI, A.; REVERS, L. F. Caracterização do perfil transcricional de genes associados à estenoespermocarpia em videira (*Vitis vinifera* L.). *In:* 9º Encontro de Iniciação Científica e 5º Encontro de Pós-Graduandos da Embrapa Uva e Vinho, Bento Gonçalves. 2011.

PARTICIPAÇÃO EM CURSOS

* I Workshop Interno De Biologia Molecular De Plantas. Universidade Federal do Rio Grande do Sul. 2014.

* Theoretical and practical aspects of gene silencing in plants. Peter Waterhouse & Rogério Margis. PPGBCM, Universidade Federal do Rio Grande do Sul. 2013.

* Sequenciamento e Análise de Fragmentos através da Eletroforese Capilar na Plataforma 310. Embrapa Uva e Vinho, Bento Gonçalves. Life Technologies Brasil. 2013.

* 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).

* Manejo e uso de recursos genéticos de uva. Embrapa Uva e Vinho, Bento Gonçalves. 2011.

* PCR em Tempo Real - Princípios e Aplicações. Life Technologies Brasil. 2011.

ATIVIDADES DESENVOLVIDAS NO PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA CELULAR E MOLECULAR - UFRGS

Período Letivo	Atividade de Ensino	Créditos	Conceito
2014/1	Atividade Didática	3	A
2014/1	Redação Científica Manuscrito	1	A
2014/1	Seminário do Centro de Biotecnologia	2	A
2014/1	Seminários de Dados Experimentais Apresentação	1	A
2014/1	Seminários de Dados Experimentais Frequência	1	A
2014/1	Tópicos Avançados	3	A
2013/2	Biologia Molecular	4	B
2013/2	Estrutura e Estratégias de Análise Genômica Vegetal	3	A
2013/1	Biologia Celular	3	A
2013/1	Curso- Theoretical and practical aspects of gene silencing in plants	2	A
2013/1	Fundamentos de Análise de Proteínas	2	A