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# Molecular characterization and *in silico* analysis of an alkaline α-galactosidase gene (*Vv-α-gal/SIP*) in grapevines (*Vitis vinifera. L*)

## [Üzüm asmasından (*Vitis vinifera.L*) elde edilen alkalen α-galaktozidaz geninin (*Vv-α-gal/SIP*) moleküler karaterizasyonu ve in *silico* analizi]*

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**ABSTRACT**

**Aim:** After identifying and isolating a grapevine alkaline α-galactosidase gene and before initializing functional genomic studies, we judged necessary to acquire minimal knowledge about this enzyme that catalyzes galactose release in the more neutral to alkaline cytoplasm. Our goal was to search and identify structural motives and domains that could provide knowledge and information about protein activity and functionality.

**Methods:** We realized a bioinformatics analysis using several tools based on the homology search, promoter analysis, in order to determine basic characteristics of this protein that could guide us in the functional characterization works. As with several bioinformatics approaches, it is the combination of tools and data resources which provides the best results.

**Results:** We discovered a new alkaline α-gal gene by using Bioinformatics tools. Vv-α-gal/SIP gene is made of 13 exons and measures 2.9 kb, it encodes a protein of 774 amino acids with a predicted molecular mass of 85 kDa. The enzyme contains several putative abiotic stress regulatory elements.

**Conclusion:** This study reports the first bioinformatic characterization of the *Vv-α-gal-SIP* gene. We investigated the possibility of predicting the function of *Vv-α-gal-SIP* only by its sequence. Several regulatory elements were predicted and described to be involved in abiotic stress signaling. The discovery of new alkaline α-Gals may contribute to important technological applications of α-Gals.

**Key Words:** α-galactosidase, Bioinformatic, seed imbibition protein.

**Conflict of Interest:** Authors have no conflict of interest.

**ÖZET**

**Amaç:** Üzüm asması alkalen α-galaktozidaz geni tanımlandıktan ve izole edildikten sonra, işlevsel genomik çalışmalara başlamadan, nötralden alkaliye dönük sitoplazmada galaktoz salınımını katalizleyen bu enzim hakkında bilgi sahibi olmak gereklidir. Amacımız proteinin aktivitesi ve işlevselliği ile ilgili bilgi sahibi olmak amacıyla yapısal bölgelerini araştırmak ve tanımlamaktır.

**Yöntem:** Proteinin temel özelliklerini belirlemek amacıyla kullanılan, homoloji araştırmaları ve promotor analizi gibi birçok yönteme dayanan biyoinformatik analiz, işlevsel karakterizasyon çalışmalarında yönlendirici olabilmektedir. Birçok biyoinformatik yaklaşımda olduğu gibi araç ve veri kaynaklarının birleşimi en iyi sonucu vermektedir.

**Bulgular:** Biyoinformatik araçlar kullanılarak yeni bir alkalen α-gals geni bulunmuştur. *Vv-α-gal/SIP* geni 2.9 kb uzunluğunda olup 13 ekzondan oluşmaktadır. Kodladığı protein 774 amino asitten oluşmaktadır ve tahmin molekül kütlesi 85 kDa'dur. Enzimin çok sayıda abiyotik stres düzenleyici eleman içerdiği düşünülmektedir.

**Sonuç:** Bu çalışma Vv-α-gal-SIP geninin ilk biyoinformatik karakterizasyonunu sunmaktadır. *Vv-α-gal-SIP* gen dizisine bakarak fonsiyonunu tahmin etme olasılığı araştırılmıştır. Birçok düzenleyici elemanın abiyotik stres sinyali ile ilişkili olduğu tahmin edilmektedir. Yeni alkalen α-Gals'in bulunması önemli teknolojik uygulamalara katkıda bulunabilir.

**Anahtar Kelimeler:** α-galaktozidaz, biyoinformatik, tohum soğurma proteini

**Çıkar Çatışması:** Yazarların çıkar çatışması bulunmamaktadır.

## Introduction

Drought and salt stresses are the major constraints limiting the productivity and geographical distribution of many plant species, including important agricultural crops. Being the most widely cultivated perennial plant, grapevine occupies a privileged position and has the greatest economic value worldwide, as well as in Tunisia. However, autochthonous Tunisian grapevine varieties exhibit enhanced osmotic tolerance and adaptive responses to stress conditions [1-2] which represent a valuable resource for studying mechanisms of adaptation to salinity and drought in *V. vinifera*. Thus understanding the molecular genetic basis of the environmental stress responses would be crucial towards improving grapevines production. We have recently identified a novel alkaline alpha galactosidase (*Vv-α-gal-SIP)* from the salt tolerant Tunisian cultivar Razegui as differentially expressed upon salt stress [3]. α-Gal is widely distributed in plant species and there are two distinct types of α-Gals which degrade oligosaccharides during seed germination and differing in their optimal pH of catalysis. While the acid α-Gal type is most likely active in the acidic environment of the vacuole and the apoplasm, the alkaline α-Gal type probably catalyzes galactose release in the more neutral or alkaline cytoplasm [4-5]. Most studies have focused on acid forms of the enzyme, which play important role in seed germination [6]. The alkaline form showed high affinity for stachyose and little activity toward raffinose compared with the acid form, for which raffinose was found to be the preferred substrate [7]. Raffinose family oligosaccharides (RFOs), particularly raffinose, play a role in the acquisition of cold tolerance in many plant species such as *Ajuga reptans* [8], *Arabidopsis thaliana* [9] and the woody plant *Viola wittrockiana* [10]. One of the suggested functions of this metabolite is the membrane protection upon cold stress. Raffinose biosynthesis is regulated by the action of two enzymes, galactinol synthase (GolS; catalyzes the first committed) and raffinose synthase [9]. Raffinose degradation proceeds by the action of α-galactosidase (α-gal; EC3.2.1.22) which catalyzes the hydrolytic cleavage of the terminal-linked moiety from Gal-containing oligosaccharides. A down-regulation of α-galactosidase enhances freezing tolerance in *Petunia* [11]. In contrast to cold stress, salt and drought stress treatment enhances the induction of the α-galactosidase expression in the salt tolerant New Zealand spinach [12] and in *Petunia* under low temperature treatment [13]. This mode of stress response was similar to the expression patterns of *Vitis vinifera* α-galactosidase (*Vv-α-gal-SIP,* GeneBank accession no. EU543561*)*. The discovery of new alkaline α-Gals may contribute to important technological applications of α-Gals. Bioinformatics tools are powerful tools in terms of leading to the discoveries and analysis of novel genes [14-15]. In fact, from the abundant gene bank data, nowadays it is possible to compare biomolecules from any organism, draw the evolution trees of different species, analyze the first or second structure of the protein, localize the protein in a cell, get post-translational modification and carry topology prediction. In this study we aimed to characterize the *Vv-α-gal-SIP* genomic organization and molecular structure using bioinformatic approach and tools. Thus we initiated and applied an *in-silico* approach to characterize a Vv-α-gal/SIP protein to deduce its functional informations (*i.e.* functional properties and mechanisms of activity regulation and expression).

## Material and methods

### *Homology search*

The search for homologous protein sequences to *Vvα-gal-SIP* was carried out using the BLASTp program [16] at NCBI (http://www.ncbi.nlm.nih.gov). Full protein sequences of α-galactosidases from different species were downloaded and then aligned using the CLUSTAL_W (DNAstar software).

### *Genomic sequence and promoter analysis*

The identification of grapevine genomic sequences was performed by consulting the following site: http://www.genoscope.cns.fr/externe/Genome Browser/Vitis/. The identification of exons from the genomic sequence was performed using GENSCAN software (http://genes.mit.edu/GENSCAN.html). The determination of the regulatory elements was performed using PLACE Signal Scan Software (http://www.dna.affrc.go.jp/htdocs/PLACE/signalup.html) [17]. Prediction of the promoter region was performed using the tools Promoter search (www.promoteScan and http://bimas.dcrt.nih.gov/molbio/proscan/) [18] and Promoter 2.0 (http://www.cbs.dtu.dk/services/promoter/) [19].

## Results and discussion

### *Genomic organization of Vv-α-gal/SIP*

Based on the recent results of *V. vinifera* Pinot Noir 12x genome annotation (Locus name GSVIVT01033305001, http://www.genoscope.cns.fr/spip/Vitis-vinifera-whole-genome.html) [20], we could analyse the genomic structure and organization of *Vv-α-gal/SIP* gene. The cDNA of *Vv-α-gal/SIP* is 2844 nucleotides long, with a 3'-untranslated region of 298 nucleotides, an open reading frame (ORF) of 2325 nucleotides and a 5'-untranslated region of 181 nucleotides (Figure 1). The *Vv-α-gal/SIP* gene is a single copy gene located on chromosome eight of the Pinot noir "40024" genome, includes 13 exons and 12 introns, and encodes for a protein of 774 amino acids with a calculated molecular mass of 84.9 kDa and an isoelectric point of 5.61.-

Comparison of the predicted protein deduced from *Vv-α-gal/SIP* cDNA with proteins from other plant species in the public databases using the BLASTp algorithm revealed identity of up to 80% to α-galactosidases from *Cucumis sativus* (GenBank accession no. ABD52008), *Cucumis melo* (GenBank accession no. AAM75140) and *Tetragonia tetragonioides* (GenBank accession no. AB434769) and putative seed imbibition proteins from other mono- and dicotyledonous plant species as well (Figure 2).

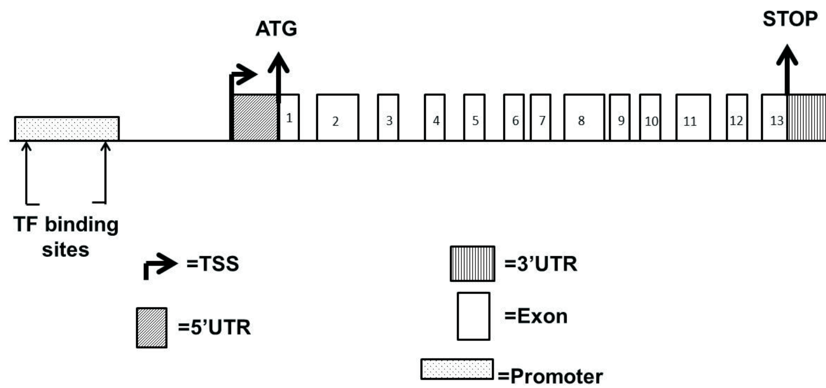


**Figure 1.** Schematic representation of the genomic organisation of *Vv-α-gal-SIP*

alkaline alpha-galactosidase

Oryza sativa Japonica Group (BAD10122.1)

Zea mays (NP_001105775.1)

Cucumis Sativus (AAZ81424.2)

Cucumis melo (AAM75140.1)

Solanum lycopersicum (AAN32954.1)

Seed imbibition protein

Oryza sativa Japonica Group (AAT77910.1)

Triticum aestivum (BA166422.1)

Hordeum vulgare subsp.vulgare (AAA32975.1)

Zea mays (NP_001147550.1)

Zea mays (NP_001105793.1)

Arabidopsis thaliana (NP_191311.1)

Arabidopsis thaliana (NP_850715.1)

Arabidopsis thaliana (NP_974451.1)

Brassica oleracea (CAA55893.1)

alkaline alpha-galactosidase/SIP

Cucumis Sativus (ABD52008.2)

Cucumis melo (AAM75140.1)

Pisum sativum (ABR19752.1)

Ricinus communis (XP_002529618.1)

Persea americana (CAB77245.1)

Vitis vinifera (ACD39775.1)

Tetragonia expansa (BAG74565.1)

0.1

**Figure 2.** Phyllogenic tree of several alkaline alpha-galactosidases, from different plants based on COBALT multiple alignments (http://www.ncbi.nlm.nih.gov/tools/cobalt/):
Alkaline alpha-galactosidase: BAD10122.1, NP_001105775.1, AAZ81424.2, AAM75139.1, AAN32954.1; Seed imbibition protein: AAT77910.1, BAI66422.1, AAA32975.1, NP_001147550.1, NP_001105793.1, NP_191311.1, NP_850715.1, NP_974451.1, CAA55893.1; Alkaline alpha-galactosidase/SIP: ABD52008.2, AAM75140.1, ABR19752.1, XP_002529618.1, CAB77245.1, ACD39775.1, BAG74565.1,

The translated protein of *Vv-α-gal/SIP* was used to query the *Vitis* genome for closely related sequences. Seven loci were identified on chromosome 5, 7, 11, 14, 17 and 19 encoding for putative proteins which showed overall scores of identity 34.1 % at the amino acid level confirming that *Vv-α-gal/SIP* on chromosome 8 is indeed a single copy gene with a genomic accession number GSVIVT00025569001.

## *Putative catalytic sites of Vv-gal/SIP*

Seed imbibition proteins have frequently been assigned a putative transgalactosylation activity due to the presence of a raffinose synthase domain (pfam05691). However growing evidence indicates that these genes encode glycosyl hydrolases possessing an alkaline alpha-galactosidase activity. Raffinose family oligosaccharides (RFO) gene members are able to use mainly stachyose and raffinose as a substrate and were related to sink activities in seed germination and fruit development [4]. We investigated the putative catalytic site(s) of the deduced protein for two well conserved motifs which have been reported [21] using *in silico* analysis among the different α-gal families (i.e., family 27 eukaryotic and plant alkaline α gals/SIPs). The presence of a conserved aspartic acid residue acts as a catalytic nucleophile to generate a covalent glycosyl-enzyme intermediate. These motives were shown to be required for enzymatic activity for the glycosyl hydrolase family 27. They were also conserved in the *Vv α-gal/SIP* predicted amino acid sequence, the domain A (DDxW) being found at amino acid position 238-241 and the domain B (KxD) being found at amino acid position 384-386 [4], thereby speculating the putative glycosyl hydrolase function of Vv α-gal/SIP. Although the potential of grapevine to synthesize RFOs like raffinose and stachyose is well documented preferentially in the context of cold hardiness [22] The role of RFO degradation *via* alkaline α-galactosidase under salt stress conditions remains elusive. Degradation of RFO may enhance the availability of specific carbohydrates under abiotic stress and may finally help the plant to survive under adverse conditions.

## *Prediction of gene regulation via transcription factors*

The identification of promoters and their regulatory elements is one of the major challenges in bioinformatics and integrates comparative, structural, and functional genomics. Many different approaches have been developed to detect conserved motifs in a set of genes that are either coregulated or orthologous. Regulation of gene expression becomes the key problem of the era of "Functional Genomics". It is known that genes are regulated mainly by means of multiple regulatory proteins transcription factors (TF), acting through specific regulatory sequences (TF binding sites) that are usually located in the proximity of the genes when constituting a promoter, or at more remote locations when acting as part of an enhancer. These TFs bind to the DNA on specific cis-acting regulatory elements (CAREs) and orchestrate the initiation of transcription, which is one of the most important control points in the regulation of gene expression. Results obtained in this study using PLACE software on the identification of transcription factors and cis acting regulatory elements are summarized in Table 1. We were able to identify several cis-acting regulatory elements. Among these TF, the ABRE motifs which are involved in gene activation of erd1 (Early Response to dehydration) and *rd22* gene (Responsive to dehydration) under water stress [23]. In addition, binding sites for MYB and MYC have also been identified in the promoter *Vvα-gal/SIP* [24]. In *Arabidopsis thaliana*, the interaction between the DRE and DREBA motifs regulates the expression of *rd29* gene in response to drought and high salt stress [25]. The CBFHV factors identified are transcriptional activators of the expression of genes regulated by water stress, such as *rab17* gene from maize [26]. In barley, these motifs allow the fixation of CBF1 and CBF2 proteins (C-repeat Binding Factor 1 and 2) involved in the response to water deficit and cold. In addition, the binding capacity of CBF2 to the target DNA sequences is regulated by temperature [27]. Proteins AtMYB1et AtMYB2 are transcriptional activators of the expression of genes inducible by ABA in water stress conditions, such as the rd22 gene in *Arabidopsis thaliana* [28-30]. The CAAT box has been reported in this study. This element was also found in *Os*-RGLP2 promoter which is located upstream of a germin like protein gene in rice [31]. The involvement of CAAT box in inducible gene expression is well established. Upstream regions of light-regulated genes in both monocots and dicots were reported to contain GATAA conserved sequence, also designated as I-box [32]. Proteins containing this motives were involved in expression of photosynthetic genes, seed storage protein genes and genes responsive to various stresses [33-35]. We have also identified the W box [(T) TGAC(C/T)] which is the binding site for members of the WRKY family of transcription factors. There is increasing evidence that W boxes are a major class of *cis* -acting elements responsible for the pathogen inducibility of many plant genes [36] also involved in senescence and trichome development [37]. W box was also found to be involved as an enhancer in the induction of salicylic acid in response to wounding and bacterial infection in *Brassica oleracea*. The TGAC element identified in our study was first reported in rice [38] and found to be involved in gibberellin signaling pathway. The TGAC motif is found as an invariant core sequence in W box which serves as a binding site for a rice transcription factor, WRKY71.

In plants, approximately 7% of the genome encodes for putative TFs [39]. The functions for most of stress responsive TF encoding genes are not fully understood. Identification and functional analysis of stress-inducible TFs will provide more information on the complex regu-

**Table 1.** Potential cis-acting and regulatory elements in Vv-α-gal-SIP promoter region

| Regulatory elements and description | Positive strand | Negative strand | Consensus sequences |
|---|---|---|---|
| **ABRELATERD1** (ABRE-like) : involved in gene expression of erd1 (early response to dehydration) | 8 | 10 | ACGT |
| CAAT **Box** | 2 | 5 | CAAT |
| **DOF** : regulation of growth and plant development | 9 | 9 | AAAG |
| **I Box** : regulation of light | 1 | 2 | GATAA |
| **MYB1AT Box** : present in promoters of dehydration responsive genes (ex RD22) | 2 | 1 | WAACCA |
| **MYB2 AT Box** : present in promoters of dehydration responsive genes (ex RD22) | 1 | 0 | YAACKG |
| **Core MYB** : fixation site of MYB proteins (ATMYB1 and ATMYB2 of Arabidopsis) involved in gene regulation in response to drought stress | 1 | 1 | CNGTTR |
| **MYCATERD1** : regulation of « erd1 »b gene under drought stress and NAC protein | 1 | 1 | CATGTG |
| **MYCATRD22 :** fixation site of **MYC in RD22 of Arabidopsis and genes regulated by ABA** | 1 | 1 | CACATG |
| **DPBFCOREDCDC3 : novel class of transcription factor** bZIP, regulating LEA (Late embryogenesis abundant gene) | 1 | 2 | ACACNNG |
| **DRE2** : regulation of rab17 gene from maize | 1 | 0 | ACCGAC |
| **DRE/CRT Motif : element responding to dehydration** | 1 | 0 | RCCGAC |
| **TATA Box** | 2 | 4 | TTATTT/ TATTTAA |
| **CAAT Box** | 2 | 5 | CAAT |
| **WRKY71OS** : regulation of PR (Pathogenesis related) genes | 5 | 5 | TGAC |
| **CBFHV: element responding to dehydration** | 1 | 0 | RYCGAC |

latory gene networks controlling stress responses, and potential tools for genetic engineering of transgenic crop plants with superior yield under stress conditions [40].

## *Future studies*

In this study, the hypothetical regulatory motifs identified in the promoter appear to be strongly involved in the regulation of abiotic stress in plants. This bioinformatics analysis can be considered as a first approach to identify a range of transcriptional regulatory motifs in a promoter. However, the identification of the promoter region, post translational modifications and transcription factors playing a role on this gene functionality and activity must be confirmed. Experimental testing of these *in silico* predictions may be a manner to increase knowledge on promoters more quickly and at a lower cost, especially for plants.

# Conclusion

The bioinformatic research on the *Vv-α-gal-SIP* represents the first *in silico* characterization of this gene that may perform important role in RFO degradation. This function was supported by *in silico* analysis for the identification of different catalytic domains which shown to be required for enzymatic activity for the glycosyl hydrolase function. *Vv-α-gal-SIP* was speculated to be involved in abiotic stress response. In fact, several regulatory elements were predicted and identified by bioinformatics tools. Most of the described cis-elements were

annotated and described to be involved in abiotic stress signaling. In silico analysis seems to be a privileged alternative to analyze simultaneously a great number of regulatory elements or promoter regions. Bioinformatics tools are powerful tools in terms of leading to the discoveries and analysis of novel genes.

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## Conflict of Interest:

Authors have no conflict of interest.

## References

[1] Jellouli N, Ben Jouira H, Skouri H, Gargouri A, Ghorbel A, Mliki A. Proteomic analysis of Tunisian grapevine cultivar Razegui under salt stress. J Plant Physiol 2008; 165:471-481.

[2] Toumi I, Gargouri M, Nouairi I, Moschou PN, Ben Salem-Fnayou A, Mliki A, Zarrouk M, Ghorbel A. Water stress induced changes in the leaf lipid composition of four grapevine genotypes with different drought tolerance. Biol Plant 2008; 52:161-164.

[3] Daldoul S, Guillaumie S, Reustle GM, Krczal G, Ghorbel A, Delrot S, Mliki A, Höfer M. Isolation and expression analysis of salt induced genes from contrasting grapevine (Vitis vinifera L.) cultivars. Plant Sci 2010; 179:489-498.

[4] Carmi N, Zhang G, Petreikov M, Gao Z, Eyal Y, Granot D, Schaffer A. Cloning and functional expression of alkaline alpha-galactosidase from melon fruit: similarity to plant SIP proteins uncovers a novel family of plant glycosyl hydrolases. Plant J 2003; 33:97–106.

[5] Lee R H, Lin M C, Chen S C. A novel alkaline alpha-galactosidase gene is involved in rice leaf senescence. Plant Mol Biol 2004; 55:281–295.

[6] Keller F, Pharr DM. In: E Zamski (ed.) Metabolism of carbohydrates in sink and sources: Galactosyl-sucrose oligosaccharides 1996; pp.157-183, Crops Marcel Dekker, New York.

[7] Gaudreault PR, Webb JA. Alkaline α-galactosidase activity and galactose metabolism in the family Cucurbitaceae. Plant Sci 1996; 45:71-75.

[8] Bachmann M, Matile P, Keller F. Metabolism of the raffinose family oligosaccharides in leaves of Ajuga reptans: cold acclimation, translocation and sink to source transition: discovery of chain elongation enzyme. Plant Physiol 1994; 105:1335–1345.

[9] Taji T, Ohsumi C, Iuchi S, Seki M, Kasuga M, Kobayashi M, Yamaguchi- Shinozaki K, Shinozaki K. Important roles of drought- and cold inducible genes for galactinol synthase in stress tolerance in Arabidopsis thaliana. Plant J 2002; 29:417–426[10] Stushnoff C, Seufferheld MJ, Creegan T. Oligosaccharides as endogenous cryoprotectants in woody plants. In P.H. Li and T.H.H. Chen, eds., Plant Cold Hardiness: Molecular Biology, Biochemistry and Physiology, 1998; pp 301–309, Plenum Press, New York.

[11] Pennycooke JC, Jones M L, Stushnoff C. Down-Regulating α-Galactosidase Enhances Freezing Tolerance in Transgenic Petunia. Plant Physiol 2003; 133:901–909.

[12] Hara M, Tokunaga K, Kuboi T. Isolation of a drought-responsive alkaline α -galactosidase gene from New Zealand spinach. Plant Biotech 2008; 25:497–501.

[13] Pennycooke JC, Vepachedu RC. Expression of an α-Galactosidase gene in Petunia is up-regulated during low-temperature deacclimation. J Am Soc Hort Sci 2004; 129: 491-496.

[14] Varisli L, Cen O. Identification and Characterization of Rat GMDS Gene by Using Bioinformatics Tools. Turk J Biochem 2005; 30:306-9.

[15] Hu Z, Chen K, Wang L, Yao Q. Identification and characterization of Bombyx mori eIF5A gene through bioinformatics approaches. In Silico Biol 2005; 5:573-80.

[16] Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol 1990; 215:403-10.

[17] Higo K, Ugawa Y, Iwamoto M, Korenaga T. Plant cis-acting regulatory DNA elements (PLACE) database, Nucl Aci Res 1999; 27:297–300.

[18] Prestridge DS. Predicting Pol II promoter sequences using transcription factor binding sites. J Mol Biol 1995; 249:923–932.

[19] Knudsen S. Promoter 2.0, for the recognition of PolII promoter sequences. Bioinformatics 1999; 15:356-361.

[20] Jaillon O, Aury JM, Noel B, Policriti A, Clepet C, Casagrande A, et al. The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla Nature 2007; 449:463-467.

[21] Blöchl A, Peterbauer T, Hofmann J, Richter A. Enzymatic breakdown of raffinose oligosaccharides in pea seeds. Planta 2008; 228:99–110.

[22] Hamman RAJ, Dami IE, Walsh TM, Stushnoff C. Seasonal Carbohydrate Changes and Cold Hardiness of Chardonnay and Riesling Grapevines. Am. J Enol Vitic 1996; 47:31-36.

[23] Simpson SD, Nakashima K, Narusaka Y, Seki M, Shinozaki K, Yamaguchi-Shinozaki K. Two different novel cis-acting elements of erd1 a clpA homologous Arabidopsis gene function in induction by dehydration stress and dark-induced senescence. Plant J 2003; 33:259-270.

[24] Choi H, Hong J, Ha J, Kang J, Kim SY. ABFs a family of ABA-responsive element binding factors. J Biol Chem 2000; 27:1723-1730.

[25] Naruska Y, Nakashima K, Shinwari Z, Sakuma Y, Furihata T, Abe H, Narusaka M, Shinozaki K, Yamaguchi-Shinozaki K. Interaction between two cis-acting elements, ABRE and DRE, in ABA dependent expression of Arabidopsis rd29A gene in response to dehydration and high-salinity stresses. Plant J 2003; 34:137-148.

[26] Kizis D, Pagès M. Maize DRE-binding proteins DBF1 and DBF2 are involved in rab17 regulation through the drought-responsive element in an ABA dependent pathway. Plant J 2002; 30:679–689.

[27] Xue GP. The DNA-binding activity of an AP2 transcriptional activator HvCBF2 involved in regulation of low-temperature responsive genes in barley is modulated by temperature. Plant J 2003; 33:373-383.

[28] Busk PK, Pages M. Regulation of abscisic acid-induced transcription. Plant Mol Biol 1998; 3:425-435.

[29] Finkelstein RR, Lynch TJ. The Arabidopsis abscisic acid response gene ABI5 encodes a basic leucine zipper transcription factor. Plant Cell 2000, 12:599-609.

[30] Abe H, Urao T, Ito T, Seki M, Shinozaki K, Yamaguchi-Shinozaki K. Arabidopsis AtMYC2 (bHLH) and AtMYB2 (MYB) Function as Transcriptional Activators in Abscisic Acid Signaling. Plant Cell 2003; 15:63-78.

[31] Mahmood T, Hyder MZ, SaqlanNaqvi SM. (2007) Cloning and sequence analysis of germin like protein gene 2 promoter from Oryza sativa L. ssp. Indica. DNA Sequence 18: 26 - 32.

[32] Terzaghi, WB, Cashmore AR. Light-regulated transcripti-

on. Annu Rev Plant Physiol Plant Mol Biol 1995; 46:445-474.

[33] Yanagisawa S, and Schmidt RJ. Diversity and similarity among recognition sequences of Dof transcription factors. Plant J 1999, 17:209-214.

[34] Kisu Y, Ono T, Shimofurutani N, Suzuki M, Esaka M. Characterization and expression of a new class of zinc finger protein that binds to silencer region of ascorbate oxidase gene. Plant Cell. Physiol 1998; 39:1054-1064.

[35] Mena M, Vicente-Carbajisa J,. Schmidt RJ, Carbonero P. An endosperm-specific Dof protein from barley, highly conserved in wheat, binds to and activates transcription from the prolamin box of a native β -hordein promoter in barley endosperm. Plant J 1998; 16:53-62.

[36] Wang Z, Yang P, Fan B, Chen Z. An oligo selection procedure for identification of sequence-specific DNA-binding activities associated with plant defense. Plant J 1998; 16:515-522.

[37] Eulgem T, Rushton PJ, Robatzek S, Somssich I E. The WRKY superfamily of plant transcription factors. Trends Plant Sci 2000; 5:199-206.

[38] Eulgem, T, Rushton P J, Schmelzer Hahlbrock E K, Somssich IE. Early nuclear events in plant defense signaling: rapid gene activation by WRKY transcription factors. EMBO J 1999; 18:4689-4699.

[39] Udvardi MK, Kakar K, Wandrey M, Montanari O, Murray J, Andriankaja A, Zhang J-Y, Benedito V, Hofer JMI, Chueng, Town C D. Legume transcription factors: global regulators of plant development and response to the environment. Plant Physiol 2007; 144:538–549.

[40] Tran L-S.P, Nakashima K, Shinozaki K, Yamaguchi-Shinozaki K. Plant gene networks in osmotic stress response: from genes to regulatory networks. Meth Enzymol 2007; 428:109–128.

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