INTEGRATION OF *OMICS* AND SYSTEM BIOLOGY APPROACHES TO STUDY GRAPEVINE (*VITIS VINIFERA* L.) RESPONSE TO SALT STRESS : A PERSPECTIVE FOR FUNCTIONAL GENOMICS - A REVIEW

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Abstract

The ability of plants to modify their behavior appropriately in response to salt stress is a major factor in their adaptation to this specific constraint. To date, environmental constraints, including salinity, become more and more unfavorable especially for glycophytes such as grapevines. Salt tolerance is a complex physiological and multigenic trait. Studying the functional networks of transcriptome, proteome and metabolome of grapevine plants subjected to salinity may help to identify candidate genes associated with salt tolerance mechanisms. Thus, the integration of *omics* tools (i.e., genomics, proteomics and metabolomics) with physiological approaches allows better understanding of the grapevine plant response and developing efficient marker-assisted selection strategies in order to generate salt stress resistant grapevine varieties. In this review, research progress in grapevine responses to salt stress is discussed, highlighting the importance of the system biology approach for identifying molecular regulatory networks leading to a better adaptation ability of grapevine to salt stress.

Key words : grapevine, *omics*, salt stress, tolerance, integrated approach

Résumé

La capacité des plantes à modifier leur comportement de façon appropriée en réponse au stress salin est un facteur fondamental de leur adaptation à cette contrainte particulière. À ce jour, les contraintes environnementales, notamment la salinité, deviennent de plus en plus défavorables pour les glycophytes et notamment pour la vigne. La tolérance au stress salin est un caractère physiologique et multigénique complexe. L'étude des réseaux fonctionnels associés au transcriptome, au protéome et au métabolome de plants de vigne soumis à une contrainte saline permet d'aider à identifier des gènes candidats associés aux mécanismes de tolérance au stress salin. Ainsi, l'intégration d'outils *omics* (génomique, protéomique et métabolomique) avec des approches physiologiques devrait permettre d'appréhender la réponse de la vigne au stress salin et de développer des stratégies efficaces de sélections assistées par marqueurs afin de générer des variétés résistantes à la salinité. Dans cette revue, les avancées dans l'étude des réponses de la vigne au stress salin sont discutées en soulignant l'importance d'approches de biologie systémique pour identifier les mécanismes moléculaires de régulation conduisant à une meilleure capacité d'adaptation de la vigne au stress salin.

Mots clés : vigne, *omics*, stress salin, tolérance, approche intégrée

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INTRODUCTION

Plants are permanently subjected to various types of stresses : osmotic, ionic, water and salt (Munns *et al.*, 2006 ; Chadli and Belkhodja, 2007). Salinity affects about 10 % of the land in the world (Cheong and Yun, 2007). The salinization registered in the arid and semi-arid ecosystems results from high soil water evaporation (Munns *et al.*, 2006), irregular and insufficient rainfall (Mezni *et al.*, 2002), as well as the use of poor quality water. Consequently, crop production with an appreciable yield becomes a challenge under these conditions. Therefore, a global understanding of plant mechanisms involved in salt stress adaptation is required. Plant response to salt stress occurs at various levels : molecular, cellular and physiological (Yamaguchi-Shinozaki *et al.*, 2002). Tolerance to abiotic stresses is a complex feature influenced by the coordinated and differential expression of a group of genes (Chen *et al.*, 2002). In general, several modifications are expected to be activated as a response to abiotic stresses (Jain *et al.*, 2001). Recently, progress has been made in the functional genomics of grapevine following the whole genome sequencing and assembling of *Vitis vinifera* PN40024 reference genome (Jaillon *et al.*, 2007). Global analyses have become possible with the development of high throughput genomic technologies which facilitated the identification of putative gene function. In parallel, methods have been developed for quantitative data acquisition : microarrays are used to quantitatively assess the transcriptome (Schena *et al.*, 1995). However, the recent advent of high throughput-based sequencing technologies has revolutionized the analysis of transcriptomes (Morozova and Marra, 2008). In fact, RNA sequencing (RNA-Seq) involves direct sequencing of complementary DNAs (cDNAs) followed by mapping of the sequencing reads to the reference genome. It allows for the precise quantification of exon expression, generating absolute rather than relative gene expression measurements, providing greater insight and accuracy than microarrays (Cloonan *et al.*, 2008 ; Mortazavi *et al.*, 2008 ; Wang *et al.*, 2009). Furthermore, it can detect and measure rare transcripts with frequencies as low as 1 to 10 RNA molecules per cell (Mortazavi *et al.*, 2008). In this context, Next-Gen sequencing technologies have emerged, such as 454 (Margulies *et al.*, 2005) or Illumina (Bennett, 2004) technologies. For example, the Illumina RNA-Seq method was successfully used by Zenoni *et al.* (2010) to analyze the global grapevine transcriptome during berry development. In proteomics, two-dimensional gels have routinely been used for proteome studies (O'Farrell, 1975). Recently, gel-free technologies have emerged, such as ICAT (Gygi *et al.*, 1999) or iTRAQ (Ross *et al.*, 2004). Metabolome studies are performed with a variety of tools such as gas chromatography or high performance liquid chromatography for the separation of the metabolites and mass spectrometry and nuclear magnetic resonance for the identification and quantification of the metabolites (Fiehn, 2002). This progress opened up a new investigation field, *omics*, from which many transcriptomic (Tattersall *et al.*, 2007 ; Daldoul *et al.*, 2010), proteomic (Vincent *et al.*, 2007 ; Jellouli *et al.*, 2008 ; Grimplet *et al.*, 2009a ; Cramer, 2010 ; Cramer *et al.*, 2013), interactomic (Carna *et al.*, 2012), metabolomic (Cramer *et al.*, 2007 ; Deluc *et al.*, 2009 ; Hochberg *et al.*, 2013) and candidate gene approaches (Hanana *et al.*, 2007 ;

Table 1. Examples of differentially expressed genes in response to abiotic stress.

Palnt Species	Gene name	abiotic Stress	Technique used	References
Capsicum annuum	*Ca LEA1*	Salt Stress, Water deficit, ABA	mRNA Differential display	(Park *et al*., 2003)
Camellia sinensis	*PR-5*	Water deficit	mRNA Differential display	(Sharma et Kumar, 2005)
Pinus pinaster Ait.	*PR-10*	Water deficit	cDNA-AFLP	(Dubos et Plomion, 2001)
Triticum durum	*AtGSK1*	Salt Stress	cDNA-AFLP	(Chen *et al*., 2003)
Zea maize	*DRE1/Rab17*	Water deficit	SSH	(Zheng *et al*., 2004)
Cicer arietinum	*MIP*	Water deficit	SSH	(Boominathan *et al.,* 2004)
Tetragonia tetragonioides	*Alpha galactosidase*	Water deficit Salt stress	mRNA Differential display	(Hara *et al.*, 2008)
Tobacco	*ASR1*	Salt stress	SSH	(Kalifa *et al*., 2004)
Vitis vinifera	*RD22*	Salt stress	Candidate gene approach	(Hanana *et al*., 2008; Jardak-Jamoussi *et al*., 2014)

ABA = Abscisic Acid ; AFLP = Amplified Fragment Length Polymorphism ; SSH = Suppressive Subtractive Hybridization

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Hanana *et al.*, 2008) were developed. The present review underlines the integration of the different *omics* tools with physiological and eco-physiological approaches and their subsequent incorporation into functional networks in order to better understand the mechanisms involved in grapevine salt tolerance.

PHYSIOLOGICAL RESPONSES OF GRAPEVINE TO SALT STRESS

Grapevine (*Vitis vinifera* L.) is a glycophyte classified as being moderately tolerant to salt (Maas, 1990). It is worth noting that salt-tolerant glycophytes are able to simultaneously limit Na^+ and Cl^- ion accumulation into the leaves and their efficient compartmentation. Thus, grapevine can avoid the toxic effects of Na^+ and Cl^- ions. Some experiments conducted by Fisarakis *et al.* (2001) on ungrafted grapevines showed that salt tolerance traits are significantly correlated with the capacity to exclude Cl^- ions from leaves and maintain a high level of growth, photosynthetic activity and stomatal conductance. Besides, Walker *et al.* (1981) demonstrated that the salt tolerance of the Sultana grapevine variety was related to its capacity to maintain cell turgor, which is associated with the decrease in leaf osmotic potential at high NaCl concentrations (>100 mM). Troncoso *et al.* (1999) demonstrated by *in vitro* studies conducted on grapevine rootstocks that their salt tolerance depends on their ability to maintain high levels of K^+ ions in their tissue. Salt tolerance in grapevine is related to an efficient sequestration of the toxic ions at the root level and, more precisely, to the restriction of their transport towards the aerial parts through the xylem (Storey *et al.*, 2003).

Short term studies have proven the toxic effect of Na^+ and Cl^- ions in grapevine grown under salt stress (Garcia and Charbaji, 1993 ; Shani *et al.*, 1993). Paradoxically, a Tunisian wild grapevine specimen called *Vitis vinifera* subsp. *sylvestris* var. 'Séjnène' adopted the sodium inclusion strategy at the leaf level concomitantly with Cl^- ions exclusion (Hamrouni *et al.*, 2011). Moreover, the wild *Vitis sylvestris* Khédhayria accession showed a good regulation of sodium transport and restriction in its leaves (Askri *et al.*, 2012). In addition, Shani and Ben-Gal (2005) reported that grapevines responded to salt constraint by a reduction in transpiration rate (Shani and Dudley, 2001) and vegetative growth due to a reduction in osmotic potential, which is considered as early response to salinity. On the other hand, foliar senescence was reported to be associated with an accumulation of Na^+ and Cl^- ions in the leaves and dependent on the duration of salt stress exposure (Shani and Ben-Gal, 2005). Other examples of specific and non-specific effects on grapevine were assessed by Walker *et al.* (1981), who reported that stomatal closure and photosynthesis are strongly affected by salinity. In Cabernet-Sauvignon cultivar, Garcia and Charbaji (1993) observed specific changes

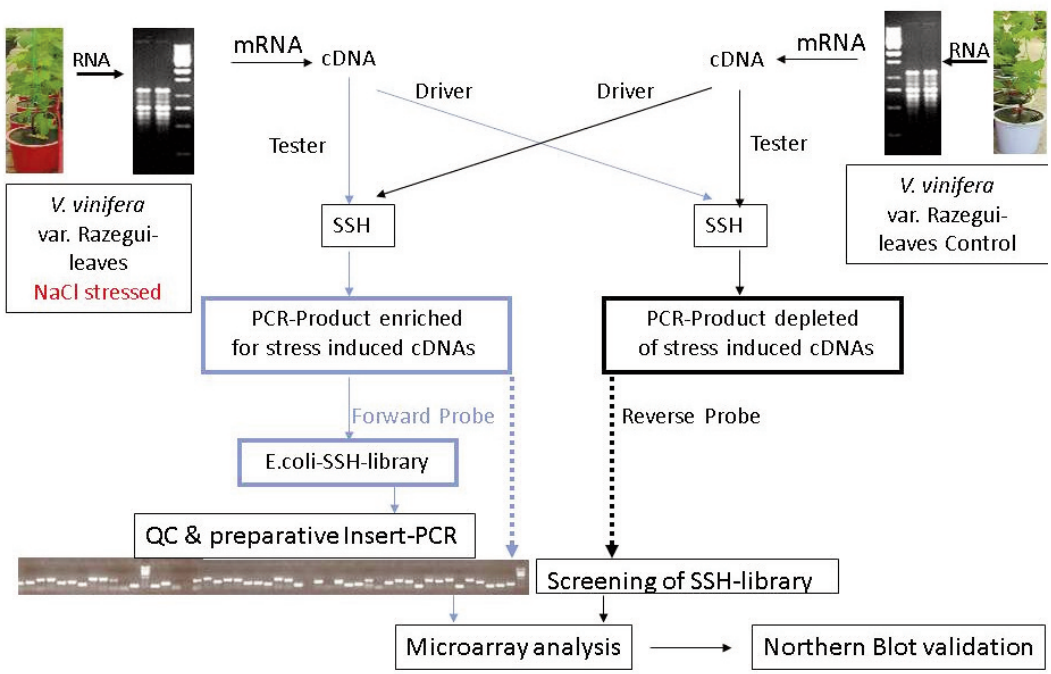


Figure 1. Strategy for identifying genes involved in salt stress response in grapevine (*Vitis vinifera* L.), as described by Daldoul *et al.* (2010). SSH = Suppressive Subtractive Hybridization ; QC-PCR = quality check PCR.

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in the Na-K balance and their antagonism under salt stress. Regarding osmolytes, salt tolerance in grapevine is related to an important reduction in sucrose and starch content along with increased levels of reducing sugars (Ashraf and Harris, 2004).

GENOMIC APPROACH AND CHARACTERIZATION OF SALT STRESS IN GRAPEVINE

Grapevine is the fourth plant, after Arabidopsis, rice and poplar, to have its genome decrypted. A highly homozygous line (97 %), PN40024, was obtained at the INRA center of Colmar (France) by cross-breeding of the Pinot Noir cultivar used for sequencing. Currently, a high coverage (12X), i.e., 12 genome equivalent, is available (http://www.genoscope.cns.fr/externe/GenomeBrowser/Vitis/;http://genomes.cribi.unipd.it/grape/). The grapevine genome is small (475 MB), diploid and composed of about 30,000 genes. Grapevine genome sequencing represents a major progress which opens up new perspectives in terms of varietal improvement and gene-function knowledge. Genome assembling and annotation allowed determining the exact physical position of all genes on the chromosomes. It also allowed to access promoter regions. Recently, grapevine genome sequencing allowed the development of high density DNA filters. These DNA microarrays (Qiagen/Operon, Combimatrix, Affymetrix, Nimble Gen) allowed (i) for the simultaneous study of the expression of thousands of genes, leading to the identification of gene networks differentially regulated during grape berry development (Goes da Silva *et al.*, 2005; Terrier *et al.*, 2005; Waters *et al.*, 2005; Grimplet *et al.*, 2007; Cramer, 2010; Guillaumie *et al.*, 2011) and (ii) investigating the molecular mechanisms of salt stress (Daldoul *et al.*, 2010). The sequencing of the grapevine genome raised numerous questions about the potential function of all the identified genes. In this respect, the integrative study of metabolite, protein and transcript profiles is recommended and could provide reliable models for prediction of the function of genes with regard to salt stress tolerance. In addition, these approaches aim also to develop efficient strategies for the selection of varieties that are more resistant to abiotic stress.

DIFFERENTIAL EXPRESSION OF GENES UNDER SALINITY

Several studies tackled the identification of genes expressed differentially in the context of abiotic stresses. Many candidate genes, which are susceptible to be associated with tolerance to salt in different species, were then identified. These studies are summarized in Table 1. The grapevine response to salt stress was also studied. In fact, Cramer *et al.* (2007) reported that the majority of genes whose expression

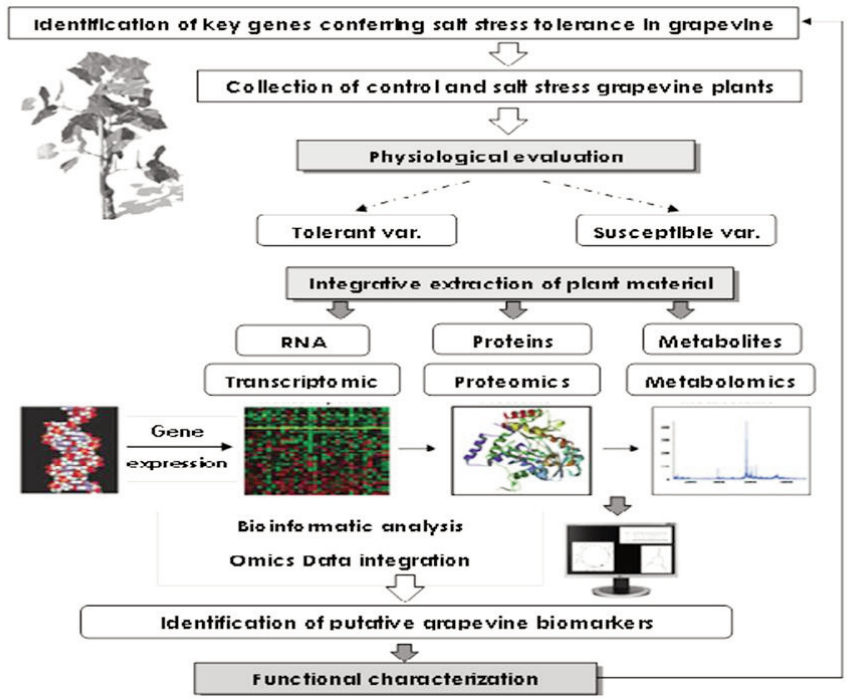


Figure 2. The *omics* cascade and System biology approach for the identification of mechanisms of salinity tolerance in grapevine.

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Table 2. Microarray analysis of salt responsive genes in grapevine leaves after 6 h and 24 h of salt stress in the contrasting cultivars Razegui (salt tolerant) and Syrah (salt sensitive).

Clone ID	Genbank accession no.	Cribi Grape Genome annotation identifier	Homology/ putative identity	Razegui - [salt stress / control]				Syrah - [salt stress / control]			
				6 h		24 h		6 h		24 h	
				Ratio	P-value	Ratio	P-value	Ratio	P-value	Ratio	P-value
SS-24H2-D1	GO238735	VIT_08s0007g08310	Alkaline alpha-galactosidase-Seed imbibition protein *(Vv-α-gal/SIP)*	3.46	5.84E-06	2.92	6.78E-03	2.17	5.84E-06	3.14	0.001185
SS-24H3-F7.1*	GH717871	VIT_11s0016g05770	Alkaline α-galactosidase2	2.8	1.75E-04	2.17	2.10E-03	-	-	1.95	2.37E-02
SS-24H3-F7.2*	GH717872	VIT_01s0150g00190	Transaldolase-like protein	2.8	1.75E-04	2.17	2.10E-03	-	-	1.95	2.37E-02
SS-24H5-D12	GH717875	VIT_12s0028g03270	Ethylene-responsive transcription factor 4	2.32	3.53E-05	2.01	0.001925	-	-	-	-
SS-24H5-G6	GH717873	VIT_04s0023g01430	Zinc finger (C2H2 type) family protein	2.87	1.60E-04	2.58	1.45E-02	-	-	2.80	5.78E-03
SS-6H1-D4	GH717874	VIT_03s0063g02110	Ubiquitin conjugating enzyme	2.45	1.74E-05	2.41	1.25E-02	-	-	3.16	6.55E-03
SS-6H3-F3	GH717876	VIT_04s0023g00580	Unknown protein	2.98	4.43E-04	3.04	4.33E-03	-	-	-	-

Genes are selected based on a statistical analysis of their differential expression, as described in Daldoul et al. (2010). These seven genes are also specifically selected because they are differentially expressed (threshold >1.9 and P-value <0.05) in at least two of the performed comparisons (6 h Raz stress vs. 6 h Raz control, 24 h Raz stress vs. 24 h Raz control, 6 h Sy stress vs. 6 h Sy control, 24 h Sy stress vs. 24 h Sy control). The grapevine genome accession number and the putative protein identity associated with these sequences are given. They are identified by using blast algorithms to compare the selected genes against the CRIBI Grape genome server and the NCBI non-redundant protein database, respectively. The following eight columns report the microarray experiment results (i.e., 6 h Raz stress vs. 6 h Raz control, 24 h Raz stress vs. 24 h Raz control, 6 h Sy stress vs. 6 h Sy control, 24 h Sy stress vs. 24 h Sy control) with relative standard deviations and P-values. Ratio values shown are expression ratios. A ratio of 2.0 indicates a two-fold up-regulation compared to control. Changes smaller than 1.90-fold are marked as (-) for non significant changes. *cDNA clones were double band; Raz = Razegui; Sy = Syrah.

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is induced by salt stress encode for transcription and protein synthesis factors. Moreover, Tattersall *et al.* (2007) were able to isolate genes that commonly respond to salt, water and cold stress. Daldoul *et al.* (2010) elaborated and screened suppressive subtractive hybridization (SSH) cDNA libraries (Figure 1). These libraries were obtained from leaves of Razegui, a Tunisian salt-tolerant variety, harvested after a short duration of salt stress (6 and 24 hours). After differential screening of the SSH libraries, various cDNA clones were sequenced. Most of the isolated expressed sequence tags (ESTs) corresponded to homologous genes previously described in other plant species as being solicited under salt stress (Daldoul *et al.*, 2012a). Moreover, the study was complemented by microarray hybridizations for a more extensive analysis of the SSH libraries and seven genes were identified as over-expressed (1.9 fold) in this salt-tolerant variety (Table 2). The functional annotation of these genes was based on sequence similarities with other heterologous genes listed and annotated in GenBank. Among the seven genes, the presence of a Zn-finger transcription factor (TF) (accession no. GH717873) was denoted by Daldoul *et al.* (2010). In this context, many studies demonstrated that the expression of Zn-finger TF is regulated differentially by various environmental stresses. Besides, these TFs allowed regulating the expression of several genes associated with the response to abiotic stresses (Kasuga *et al.*, 1999; Kim *et al.*, 2001). In Cabernet Sauvignon, the expression of a gene coding for the transcription factor « ethylene-responsive transcription factor 4 » also proved to be induced by salt and water deficit (Cramer *et al.*, 2005). The early induction of this transcript (accession no. GH717875) was also observed in the tolerant variety Razegui (Daldoul *et al.*, 2010). The transaldolase protein (accession no. GH717872) that was isolated from the SSH libraries was also identified through screening the normalized library constructed from rice seedlings subjected to water deficit (Reddy *et al.*, 2002). However, the role of this protein remains unknown. Recently, the functional characterization of an alkaline α-galactosidase of rice (Lee *et al.*, 2009, accession no. Q8W2G5) revealed that this enzyme is involved in the hydrolysis of the glycolipid digalactosyldiacylglycerol (DGDG), hence releasing monogalactosyl diglyceride (MGDG) molecules (Lee *et al.*, 2004; Lee *et al.*, 2009). DGDG was largely described in the context of abiotic stress response and more specifically as a response to water deficit in various species such as *Arabidopsis thaliana* (Gigon *et al.*, 2004) and *Vitis vinifera* (Toumi *et al.*, 2008). These lipid molecules can also be a second messenger involved in the signal transduction pathway as a response to salt stress (Munnik *et al.*, 1998; Lee *et al.*, 2009). Studies conducted by Daldoul *et al.* (2010) demonstrated that the closest homologue of *Vv-α-gal/SIP* (accession no. GO238735) was the α-galactosidase alkaline from melon. The alkaline activity of this enzyme was extensively characterized by Carmi *et al.* (2003). Based on this sequence homology, *Vv-α-gal/SIP* may have the same function and the expression of the gene coding for this enzyme is speculated to be involved in abiotic stress tolerance mechanisms (Daldoul *et al.*, 2012b). Bioinformatic analysis of the *Vv-α-gal/SIP* gene showed several regulatory elements involved in abiotic stress signaling (Daldoul *et al.*, 2012c). The differential expression of this gene was also observed in *Vitis sylvestris* grapevines and preferentially in the salt-tolerant accession Khédhayria (Askri *et al.*, 2012). In this way, the *Vv-α-gal/SIP* gene could be used as a selection marker for tolerance to salt stress in grapevine. The screening results of the salt-stressed SSH libraries also identified the cDNA clone encoding for MAP kinase (accession no. GH717878.1). Interestingly, *VvMAP* kinase transcript showed a differential expression towards salt and drought treatment in the salt-tolerant cultivar Razegui (Daldoul *et al.*, 2012d). The *VvMAP* kinase gene could be classified as an osmotic stress responsive gene as its expression was induced by salinity and drought (Daldoul *et al.*, 2012e). This transcript provides the basis for future research on the diverse signaling pathways mediated by MAPKs in grapevine.

The above work provided useful candidate genes for genetic improvement in grapevines and suggested that the dynamic expression changes observed reflect the integrative control and transcriptptional regulation networks in this species.

Based on the hypothesis that a difference in gene regulation is an indicator of an adaptive response, many differentially-expressed genes under abiotic stress conditions were used to transform model or agronomically-interesting plants (Zhang and Blumwald, 2001; Figueras *et al.*, 2004; Mukhopadhyay *et al.*, 2004) in order to improve abiotic stress tolerance and productivity. In this context, a salt-induced *VvRD22* gene from grapevine was recently characterized to be involved in salt stress tolerance in tobacco plants (Jardak-Jamoussi *et al.*, 2014).

INTEGRATION OF *OMICS* APPROACHES TO IDENTIFY MOLECULAR REGULATORY NETWORKS OF SALT STRESS TOLERANCE IN GRAPEVINE

The plant response to salt stress corresponds to a multigenic character (Pardo, 2010). It is therefore

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necessary to have reliable markers that would characterize tolerance behaviors. Furthermore, with the emergence of genome sequencing, organisms are now seen as complex interactive systems. Various transcriptomic approaches have been developed in grapevines especially after the completion of its genome sequencing (Jaillon *et al.*, 2007). The development of biotechnological tools dedicated to transcriptomic analysis in grapevine constitutes a valuable opportunity to elucidate or dissect the basis of salt stress tolerance at the molecular level (Cramer *et al.*, 2005). It also allows the analysis of genes induced by salt stress (Cramer, 2010 ; Daldoul *et al.*, 2010). However, these approaches, essentially based on the synthesis of RNA transcripts, are insufficient to dissect the molecular mechanisms of tolerance to salt stress. In fact, the information at the mRNA level provides an idea about the regulation of gene expression in a cell but must be combined with data at the protein level (i.e., proteomics data), which are often more informative. Knowing when and where a gene is transcribed then translated into a protein constitutes an important clue to determine its biological function (Bouchez and Höfte, 1998). Bulk analysis of coding transcripts requires a comparison with the corresponding proteins for better characterization. Accordingly, to compare the expression of a gene and its protein level within the same organism and in contrasting physiological states can provide response elements on the function of transcripts and their effective involvement in a particular character. Gygi *et al.* (1999) reported that the abundance of transcripts is not a criterion of prediction of the abundance of cellular proteins. Paradoxically, the work of Futcher *et al.* (1999) demonstrated a good correlation between the transcripts and their corresponding proteins. It appears, therefore, that there exists in certain cases a partial correlation between the level of transcripts and their corresponding proteins (Greenbaum *et al.*, 2002). Protein investigations in grapevine using proteomic techniques have significantly improved our knowledge in this field (Giribaldi and Giuffrida, 2010). However, Carna *et al.* (2012) highlighted that interactomics, a discipline that describes the whole set of molecular interactions in cells, must be combined with proteomics. In fact, interactomes (i.e., the full set of protein family interactions within a proteome) of different species can provide information about the evolutionary mechanisms leading to organism diversity. The study of grapevine and five other species interactomes (yeast, *Drosophila*, worm, *Arabidopsis* and human) allowed the identification of 16 protein families that were similar and had the same function (Repka and Baumgartnerova, 2008). Thus, comparative interactomics provides molecular evidence that grapevine cells were originated from heritable alterations in the pattern of gene expression. On the other hand, the metabolite profile does not tell exactly whether the related metabolic pathway is up- or down-regulated since both up-regulation of upstream reaction and down-regulation of downstream reactions can lead to the accumulation of a metabolite. This can be solved by comparing the metabolomic data with those from transcriptomic, or proteomic, and enzyme activity analysis (Cramer *et al.*, 2011). Thus, the integration of transcriptomic, proteomic, interactomic and metabolomic approaches, which is referred to as « system biology » (Figure 2), remains a necessity in order to better understand the molecular mechanisms of abiotic stress tolerance in plants (Cramer *et al.*, 2011). In this regard, there is a need for common databases and bioinformatic tools to allow a wide and deep use of these important resources. In this way, several databases were built, such as VitisExpDB (Doddapaneni *et al.*, 2008) which provides grapevine genomic resources for the functional analysis, annotation and identification of genes. The VitisNet database (www.sdstate.edu/ps/research/vitis/pathways.cfm) focuses on the molecular networks occurring in grapevine. This database can be used to visualize changes in transcriptome, proteome and metabolome within molecular networks during a given experiment (Grimplet *et al.*, 2009b ; Grimplet *et al.*, 2012). Although networks in systems biology might not completely represent the dynamic biological system, the proper application of these techniques will provide significant insight into the mechanisms of plant abiotic stress (Gupta *et al.*, 2013). The development of grapevine bioinformatic tools could allow transcript, protein and metabolite *omics* data to be displayed on molecular pathways. The integration of the grapevine databases with a system biology approach will greatly facilitate the understanding of gene function and improve production efficiency under adverse environmental conditions. Integrative functional genomics has successfully demonstrates connections between genes and metabolites. Moreover, the presence/absence and relative accumulation of certain metabolites along with gene expression data provides accurate markers for tolerant crop selection in breeding programs (Arbona *et al.*, 2013). The combination of these *omics* analyses was used to confirm that water deficit up-regulated the phenylpropanoid pathway in Cabernet Sauvignon berry skin in a tissue-specific manner (Grimplet *et al.*, 2007 ; Deluc *et al.*, 2009 ; Grimplet *et al.*, 2009a). In addition, Zamboni *et al.* (2010) identified stage-specific functional networks of linked transcripts, proteins and metabolites, providing important insights into the key molecular processes

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that determine wine quality. Thus, this multi-targeted approach could lead to the development of efficient strategies of marker-assisted selection of resistant varieties. The integration of these strategies also allowed the development of models that could predict gene function in plants (Cramer, 2010). In this way, several studies were undertaken to investigate and understand the functions of salt stress proteins in grapevines via transcriptomic (Cramer *et al.*, 2007 ; Daldoul *et al.*, 2010), proteomic (Vincent *et al.*, 2007 ; Jellouli *et al.*, 2008) and metabolic analysis (Cramer *et al.*, 2007). All of these differential approaches have opened up many application perspectives in terms of plant improvement and better tolerance to non-favorable environmental conditions.

GRAPEVINE FUNCTIONAL GENOMICS

The present challenge aims to explore the biological function of candidate genes and proteins which may be used to improve the tolerance phenotype in grapevines. In this perspective, transient expression systems by agro-infiltration (Zottini *et al.*, 2008) were developed in grapevine as a rapid way to evidence protein activity. These transient systems of expression may contribute to study grapevine gene function at various levels : cellular, tissue, and even whole-plant (Vidal *et al.*, 2010). In grapevine, many biological (via *Agrobacterium*) and physical techniques (biolistics, electroporation and protoplasts) were developed to transfer genes and offer even more basic approaches for functional genomics. Therefore, the first transgenic grapevines were obtained by Mullins *et al.* (1990). Several transgenic lines of different genotypes of *Vitis* were successfully obtained from embryogenic tissue following inoculation with *Agrobacterium tumefaciens* (Li *et al.*, 2006) or by bombarding with DNA-coated microprojectiles (Vidal *et al.*, 2006). Although no functional characterization of genes related to salt stress tolerance has yet been reported in grapevine, the transgenic approach is of capital importance to explore the function of these genes.

CONCLUSION

Most of the morphological and physiological features that make a plant tolerant to an abiotic constraint have been identified. However, the mechanisms that are implemented remains unclear with regard to grapevine, in particular. As stated in this review, the nature and complexity of salt stress responses in grapevines supports the use of global, integrative and multidisciplinary approaches to understand the different levels of regulation of salinity responses. The combination of physiological, transcriptomic, proteomic, interactomic and metabolomic approaches facilitate the identification of genes that are mostly involved in tolerance to salt stress. The large number of publications on grapevine physiology, transcriptome, proteome, and metabolome profiling published over the last few years has highlighted that in addition to its economic role, grapevine is considered more and more as a model plant, especially after the sequencing of its entire genome. Hence, *omics* and system biology approaches will open up large application perspectives in terms of viticulture improvement/development of plants with a better resistance/tolerance to salt stress. Therefore, understanding the function of genes remains a major challenge for post-genomic research.

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REFERENCES

Arbona V., Manzi M., de Ollas C. and Gómez-Cadenas A., 2013. Metabolomics as a tool to investigate abiotic stress tolerance in plants. *Int. J. Mol. Sci.*, **14**, 4885-4911.

Ashraf M. and Harris P.J.C., 2004. Potential biochemical indicators of salinity tolerance in plants. *Plant Sci.*, **166**, 3-16.

Askri H., Daldoul S., Ben Ammar A., Rejeb S., Jardak R., Rejeb M.N., Mliki A. and Ghorbel A., 2012. Short-term response of wild grapevines (*Vitis vinifera* L. *ssp. sylvestris*) to NaCl salinity exposure : changes of some physiological and molecular characteristics. *Acta Physiol. Plant.*, **34**, 957-968.

Bennett S., 2004. Solexa Ltd. *Pharmacogenomics*, **5**, 433-438.

Boominathan P., Shukla R., Kumar A., Manna D., Negi D., Verma P.K. and Chattopadhyay D., 2004. Long term transcript accumulation during the development of dehydration adaptation in *Cicer arietinum*. *Plant Physiol.*, **135**, 1608-1620.

Bouchez D. and Hofte H., 1998. Functional genomics in plants. *Plant Physiol.*, **118**, 725-732.

Carmi N., Zhang G., Petreikov M., Gao Z., Eyal Y., Granot D. and Schaffer A.A., 2003. 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.*, **33**, 97-106.

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Čarná M., Repka V. and Šturdík E., 2012. Proteomics and interactomics in grapevine : the next level in the study. *Acta Chim. Slov.*, **5**, 211-219.

Chadli R. and Belkhodja M., 2007. Réponses minérales chez la fève (*Vicia faba* L.) au stress salin. *Eur. J. Sci. Res.*, **18**, 645-654.

Chen G.P., Ma W.S., Huang Z.J., Xu T., Xue Y.B. and Shen Y.Z., 2003. Isolation and characterization of *TaGSK1* involved in wheat salt tolerance. *Plant Sci.*, **165**, 1369-1375.

Chen W., Provart N.J., Glazebrook J., Katagiri F., Chang H.S., *et al.*, 2002. Expression profile matrix of Arabidopsis transcription factor genes suggests their putative functions in response to environmental stresses. *Plant Cell*, **14**, 559-574.

Cheong M.S. and Yun D.J., 2007. Salt-stress signalling. *J. Plant Biol*., **50**, 148-155.

Cloonan N., Forrest A.R., Kolle G., Gardiner B.B., Faulkner G.J. *et al.*, 2008. Stem cell transcriptome profiling via massive-scale mRNA sequencing. *Nat. Methods*, **5**, 613-619.

Cramer G.R., Cushman J.-C., Schooley D.A., Quilici D., Vincent D., Bohlman M.C., Ergul A., Tattersall E.A.R., Tillett R., Evans J., Delacruz R., Schlauch K. and Mendes P., 2005. Progress in bioinformatics - the challenge of integrating transcriptomic, proteomic and metabolomic information. *Acta Hort.*, **689**, 417-425.

Cramer G.R., Ergül A., Grimplet J., Tillett R.L., Tattersall E.A.R., Bohlman M.C., Vincent D., Sonderegger J., Evans J., Osborne C., Quilici D., Schlauch K.A., Schooley D.A. and Cushman J.-C., 2007. Water and salinity stress in grapevines : early and late changes in transcript and metabolite profiles. *Funct. Integr. Genomics*, **7**, 111-134.

Cramer G.R., 2010. Abiotic stress and plant responses from the whole vine to the genes. *Aust. J. Grape Wine Res.*, **16**, 86-93.

Cramer G.R., Urano K., Delrot S., Pezzotti M. and Shinozaki K., 2011. Effects of abiotic stress on plants : a systems biology perspective. *BMC Plant Biol.*, **11**, 163.

Cramer G.R., Van Sluyter S.C., Hopper D.W., Pascovici D., Keighley T. and Haynes P.A., 2013. Proteomic analysis indicates massive changes in metabolism prior to the inhibition of growth and photosynthesis of grapevine (*Vitis vinifera* L.) in response to water deficit. *BMC Plant Biol.*, **13**, 49.

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

Daldoul S., Mliki A. and Höfer M.U., 2012-a. Suppressive subtractive hybridization method analysis and its application to salt stress in grapevine (*Vitis vinifera* L.). *Russ. J. Genet. (Genetika)*, **48**, 179-185.

Daldoul S., Toumi I., Reustle G.M., Krczal G., Ghorbel A., Mliki A. and Höfer M.U., 2012-b. Molecular cloning and characterisation of a cDNA encoding a putative alkaline alpha-galactosidase from grapevine (*Vitis vinifera* L.) that is differentially expressed under osmotic stress. *Acta Physiol. Plant.*, **34**, 891-903.

Daldoul S., Hanana M. and Mliki A., 2012-c. Molecular characterization and *in silico* analysis of an alkaline a-galactosidase gene (*Vv-α-gal/SIP*) in grapevines (*Vitis vinifera*. L). *Turk. J. Biochem.*, **37**, 368-374.

Daldoul S., Hoefer M.U. and Mliki A., 2012-d. Osmotic stress induces the expression of *VvMAP* kinase gene in grapevine (*Vitis vinifera* L.). *J. Bot.*, doi : 10.1155/2012/737035.

Daldoul S., Höfer M.U., Ghorbel A. and Mliki A., 2012-e. Differential expression of osmotic stress-associated ESTs in grapevine cultivars (*Vitis vinifera*. L) cultivated under salt and drought stresses. In : *Grapevines : Varieties, Cultivation and Management*. Szabo P.V. and Shojania J. (Eds.), Nova Science, pp. 169-183.

Deluc L.G., Quilici D.R., Decendit A., Grimplet J., Wheatley M.D., Schlauch K.A., Merillon J.M., Cushman J.-C. and Cramer G.R., 2009. Water deficit alters differentially metabolic pathways affecting important flavor and quality traits in grape berries of Cabernet Sauvignon and Chardonnay. *BMC Genomics*, **10**, 212.

Doddapaneni H., Lin H., Walker M.A., Yao J. and Civerolo E.L., 2008. VitisExpDB : a database resource for grape functional genomics. *BMC Plant Biol.*, **8**, 23, doi : 10.1186/1471-2229-8-23.

Dubos C. and Plomion C., 2001. Drought differentially affects expression of a PR-10 protein, in needles of maritime pine (*Pinus pinaster* Ait.) seedlings. *J. Exp. Bot.*, **52**, 1143-1144.

Fiehn O., 2002. Metabolomics – the link between genotypes and phenotypes. *Plant Mol. Biol.*, **48**, 155-171.

Figueras M., Pujal J., Saleh A., Savé R., Pagès M. and Goday A., 2004. Maize Rab17 overexpression in *Arabidopsis* plants promotes osmotic stress tolerance. *Ann. Appl. Biol.*, **144**, 251-257.

Fisarakis I., Chartzoulakis K. and Stavrakas D., 2001. Response of Sultana vines (*V. vinifera* L.) on six rootstocks to NaCl salinity exposure and recovery. *Agric. Water Manage.*, **51**, 13-27.

Futcher B., Latter G.I., Monardo P., McLaughlin C.S. and Garrels J.I., 1999. A sampling of the yeast proteome. *Mol. Cell Biol.*, **19**, 7357-7368.

Garcia M. and Charbaji T., 1993. Effect of sodium chloride salinity on cation equilibria in grapevine. *J. Plant Nutr*., **16**. 2225-2237.

Gigon A., Matos A.R., Laffray D., Zuily-Fodil Y. and Pham-Thi A.T., 2004. Effect of drought stress on lipid

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metabolism in the leaves of *Arabidopsis thaliana* (Ecotype Columbia). *Ann. Bot.*, **94**, 345-351.

Giribaldi M. and Giuffrida M.G., 2010. Heard it through the grapevine : proteomic perspective on grape and wine. *J. Proteomics*, **73**, 1647-1655.

Goes da Silva F., Iandolino A., Al-Kayal F., Bohlmann M.C., Cushman M.A., *et al.*, 2005. Characterizing the grape transcriptome. Analysis of expressed sequence tags from multiple Vitis species and development of a compendium of gene expression during berry development. *Plant Physiol.*, **139**, 574-597.

Greenbaum D., Jansen R. and Gerstein M., 2002. Analysis of mRNA expression and protein abundance data : an approach for the comparison of the enrichment of features in the cellular population of proteins and transcripts. *Bioinformatics*, **18**, 585-596.

Grimplet J., Deluc L.G., Tillett R.L., Wheatley M.D., Schlauch K.A., Cramer G.R. and Cushman J.-C., 2007. Tissue-specific mRNA expression profiling in grape berry tissues. *BMC Genomics*, **8**, 187, doi : 10.1186/1471-2164-8-187.

Grimplet J., Wheatley M.D., Jouira H.B., Deluc L.G., Cramer G.R. and Cushman J.-C., 2009-a. Proteomic and selected metabolite analysis of grape berry tissues under well-watered and water-deficit stress conditions. *Proteomics*, **9**, 2503-2528.

Grimplet J., Cramer G.R., Dickerson J.A., Mathiason K., Van Hemert J. and Fennell A.Y., 2009-b. VitisNet : "Omics" integration through grapevine molecular networks. *PLoS ONE*, **4**, e8365.

Grimplet J., Van Hemert J., Carbonell-Bejerano P., Díaz-Riquelme J., Dickerson J., Fennell A., Pezzotti M. and Martínez-Zapater J.M., 2012. Comparative analysis of grapevine whole-genome gene predictions, functional annotation, categorization and integration of the predicted gene sequences. *BMC Res. Notes*, **5**, 213.

Guillaumie S., Fouquet R., Kappel C., Camps C., Terrier N., Moncomble D., Dunlevy J.D., Davies C., Boss P.K. and Delrot S., 2011. Transcriptional analysis of late ripening stages of grapevine berry. *BMC Plant Biol.*, **11**, 165.

Gupta B., Sengupta A., Saha J. and Gupta K., 2013. Plant abiotic stress : 'Omics' approach. *J. Plant Biochem. Physiol.*, **1**, 3.

Gygi S.P., Rochon Y., Franza B.R. and Aebersold R., 1999. Correlation between protein and mRNA abundance in yeast. *Mol. Cell. Biol.*, **19**, 1720-1730.

Hamrouni L., Hanana M., Abdelly C. and Ghorbel A., 2011. Exclusion du chlorure et inclusion du sodium : deux mécanismes concomitants de tolérance à la salinité chez la vigne sauvage *Vitis vinifera* subsp. *sylvestris* (var. 'Séjnène'). *Biotechnol. Agron. Soc. Environ.*, **15**, 387-400.

Hanana M., Cagnac O., Yamaguchi T., Hamdi S., Ghorbel A. and Blumwald E., 2007. A grape berry (*Vitis vinifera* L.) cation/proton antiporter is associated with berry ripening. *Plant Cell Physiol.,* **48**, 804-811.

Hanana M., Deluc L., Fouquet R., Daldoul S., Léon C., Barrieu F., Ghorbel A., Mliki A. and Hamdi S., 2008. Identification et caractérisation d'un gène de réponse à la déshydratation *rd22* chez la vigne (*Vitis vinifera* L.). *C.R. Biol.*, **331**, 569-578.

Hara M., Tokunaga K. and Kuboi T., 2008. Isolation of a drought-responsive alkaline α-galactosidase gene from New Zealand spinach. *Plant Biotech.*, **25**, 497-501.

Hochberg U., Degu A., Toubiana D., Gendler T., Nikoloski Z., Rachmilevitch S. and Fait A., 2013. Metabolite profiling and network analysis reveal coordinated changes in grapevine water stress response. *BMC Plant Biol.*, **13,** 184.

Jaillon O., Aury J.M., Noel B., Policriti A., Clepet C., *et al.*, 2007. The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. *Nature*, **449**, 463-467.

Jain A.K., Basha S.M. and Holbrook C.C., 2 001. Identification of drought-responsive transcripts in peanut (*Arachis hypogaea* L.). *Electron. J. Biotechnol.*, **4**, ISSN : 0717-3458.

Jardak-Jamoussi R., Elabbassi M.M., Ben Jouira H., Hanana M., Zoghlami N., Ghorbel A. and Mliki A., 2014. Physiological responses of transgenic tobacco plants expressing the dehydration-responsive *RD22* gene of *Vitis vinifera* to salt stress. *Turk. J. Bot.*, **38**, 268-280, doi : 10.3906/bot-1301-53.

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

Kalifa Y., Perlson E., Gilad A., Konrad Z., Scolnik P.A. and Bar-Zvi D., 2004. Over-expression of the water and salt stress-regulated *Asr1* gene confers an increased salt tolerance. *Plant Cell Environ.*, **27**, 1459-1468.

Kasuga M., Liu Q., Miura S., Yamaguchi-Shinozaki K. and Shinozaki K., 1999. Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcription factor. *Nature Biotechnol.*, **17**, 287-291.

Kim J.-C., Lee S.H., Cheong Y.H., Yoo C.M., Lee S.I., Chun H.J., Yun D.J., Hong J.-C., Lee S.Y., Lim C.O. and Cho M.J., 2 001. A novel cold-inducible zinc finger protein from soybean, SCOF-1, enhances cold tolerance in transgenic plants. *Plant J.*, **25**, 247–259.

Lee R.H., Lin M.C. and Chen S.C.G., 2004. A novel alkaline α-galactosidase gene is involved in rice leaf senescence. *Plant Mol. Biol.*, **55**, 281-295.

Lee R.H., Hsu J.H., Huang H.J., Lo S.F. and Chen S.C.G., 2009. Alkaline α-galactosidase degrades thylakoid

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membranes in the chloroplast during leaf senescence in rice. *New Phytol.*, **184**, 596-606.

Li Z.T., Dhekney S., Dutt M., van Aman M., Tattersall J., Kelley K.T. and Gray D.J., 2006. Optimizing *agrobacterium*-mediated transformation of grapevine. *In Vitro Cell. Dev. Biol. Plant*, **42**, 220-227.

Maas E.V., 1990. Crop salt tolerance. In : *Agricultural Salinity Assessment and Management*. Tanji K.K. (Ed.), ASCE Manuals and Reports on Engineering 71, New York, pp. 262-304.

Margulies M., Egholm M., Altman W.E., Attiya S., Bader J.S., *et al.*, 2005. Genome sequencing in microfabricated high-density picolitre reactors. *Nature*, **437**, 376-380.

Mezni M., Albouchi A., Bizid E. and Hamza M., 2002. Effet de la salinité des eaux d'irrigation sur la nutrition minérale chez trois variétés de luzerne pérenne (*Medicago sativa*). *Agronomie*, **22**, 283-291.

Morozova O. and Marra M.A., 2008. Applications of next-generation sequencing technologies in functional genomics. *Genomics*, **92**, 255-264.

Mortazavi A., Williams B.A., McCue K., Schaeffer L. and Wold B., 2008. Mapping and quantifying mammalian transcriptomes by RNA-Seq. *Nat. Methods*, **5**, 621-628.

Mukhopadhyay A., Vij S. and Tyagi A.K., 2004. Overexpression of a zinc-finger protein gene from rice confers tolerance to cold, dehydration, and salt stress in transgenic tobacco. *Proc. Natl. Acad. Sci. USA*, **101**, 6309-6314.

Mullins M.G., Tang F.C.A. and Facciotti D., 1990. *Agrobacterium*-mediated genetic transformation of grapevines : transgenic plants of *Vitis rupestris* SCHEELE and buds of *Vitis vinifera* L. *Nat. Biotechnol.*, **8**, 1041-1045.

Munnik T., van Himbergen J.A.J., Ter Riet B., Braun F.J., Irvine R.F., van den Ende H. and Musgrave A., 1998. Detailed analysis of the turnover of polyphosphoinositides and phosphatidic acid upon activation of phospholipases C and D in *Chlamydomonas* cells treated with non-permeabilizing concentrations of mastoparan. *Planta*, **207**, 133-145.

Munns R., James R.A. and Lauchli A., 2006. Approaches to increasing the salt tolerance of wheat and other cereals. *J. Exp. Bot.*, **57**, 1025-1043.

O'Farrell P.H., 1975. High resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem.*, **250**, 4007-4021.

Pardo J.M., 2010. Biotechnology of water and salinity stress tolerance. *Curr. Opin. Biotechnol.*, **21**, 185-196.

Park J.A., Cho S.K., Kim J.E., Chung H.S., Hong J.P., Hwang B., Hong C.B. and Kim W.T., 2003. Isolation of cDNAs differentially expressed in response to drought stress and characterization of the *Ca-LEAL1* gene encoding a new family of atypical LEA-like protein homologue in hot pepper (*Capsicum annuum* L. cv. Pukang). *Plant Sci.*, **165**, 471-481.

Reddy A.R., Ramakrishna W., Chandra Sekhar A., Ithal N., Babu P.R., Bonaldo F.M., Soares B. and Bennetzen J.L., 2002. Novel genes are enriched in normalized cDNA libraries from drought-stressed seedlings of rice (*Oryza sativa* L. subsp. *indica* cv Nagina 22). *Genome*, **45**, 204-211.

Repka V. and Baumgartnerova I., 2008. Grapevine habituation : understanding of factors that contribute to neoplastic transformation and somaclonal variation. *Acta Agron. Hung.*, **56**, 399-408.

Ross P.L., Huang Y.N., Marchese J.N., Williamson B., Parker K., *et al.*, 2004. Multiplexed protein quantitation in *Saccharomyces cerevisiae* using amine-reactive isobaric tagging reagents. *Mol. Cell. Proteomics*, **3**, 1154-1169.

Schena M., Shalon D., Davis R.W. and Brown P.O., 1995. Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science*, **270**, 467-470.

Shani U., Waisel Y., Eshel A., Xue S. and Ziv G., 1993. Responses to salinity of grapevine plants with split root systems. *New Phytol.*, **124**, 695-701.

Shani U. and Dudley L.M., 2001. Field studies of crop response to water and salt stress. *Soil Sci. Soc. Am. J.*, **65**, 1522-1528.

Shani U. and Ben-Gal A., 2005. Long-term response of grapevines to salinity : osmotic effects and ion toxicity. *Am. J. Enol. Vitic.*, **56**, 148-154.

Sharma P. and Kumar S., 2005. Differential display-mediated identification of three drought-responsive expressed sequence tags in tea [*Camellia sinensis* (L.) O. Kuntze]. *J. Biosci.*, **30**, 231-235.

Storey R., Schachtman D.P. and Thomas M.R., 2003. Root structure and cellular chloride, sodium and potassium distribution in salinized grapevines. *Plant Cell. Environ.*, **26**, 789-800.

Tattersall E.A.R., Grimplet J., DeLuc L., Wheatley M.D., Vincent D., Osborne C., Ergül A., Lomen E., Blank R.R., Schlauch K.A., Cushman J.-C. and Cramer G.R., 2007. Transcript abundance profiles reveal larger and more complex responses of grapevine to chilling compared to osmotic and salinity stress. *Funct. Integr. Genomics*, **7**, 317-333.

Terrier N., Glissant D., Grimplet J., Barrieu F., Abbal P., Couture C., Ageorges A., Atanassova R., Léon C., Renaudin J.P., Dédaldéchamp F., Romieu C., Delrot S. and Hamdi S., 2005. Isogene specific oligo arrays reveal multifaceted changes in gene expression during grape berry (*Vitis vinifera* L.) development. *Planta*, **222**, 832-847.

Toumi I., Gargouri M., Nouairi I., Moschou P.N., Ben Salem-Fnayou A., Mliki A., Zarrouk M. and Ghorbel A., 2008. Water stress induced changes in the leaf lipid composition of four grapevine genotypes

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with different drought tolerance. *Biol. Plant.*, **52**, 161-164.

Troncoso A., Matte C., Cantos M. and Lavee S., 1999. Evaluation of salt tolerance of *in vitro*-grown grapevine rootstock varieties. *Vitis*, **38**, 55-60.

Vidal J.R., Kikkert J.R., Donzelli B.D., Wallace P.G. and Reisch B.I., 2006. Biolistic transformation of grapevine using minimal gene cassette technology. *Plant Cell Rep.*, **25**, 807-814.

Vidal J.R., Gomez C., Cutanda M.C., Shrestha B.R., Bouquet A., Thomas M.R. and Torregrosa L., 2010. Use of gene transfer technology for functional studies in grapevine. *Aust. J. Grape Wine Res.*, **16**, 138-151, doi: 10.1111/j.1755-0238.2009.00086.x.

Vincent D., Ergul A., Bohlman M.C., Tattersall E.A.R., Tillett R.L., Wheatley M.D., Woolsey R., Quilici D.R., Joets J., Schlauch K., Schooley D.A., Cushman J.-C. and Cramer G.R., 2007. Proteomic analysis reveals differences between *Vitis vinifera* L. cv. Chardonnay and cv. Cabernet-Sauvignon and their responses to water deficit and salinity. *J. Exp. Bot.*, **58**, 1873-1892.

Walker R.R., Torokfalvy E., Scott N.S. and Kriedemann P.E., 1981. An analysis of photosynthetic response to salt treatment in *Vitis vinifera*. *Aust. J. Plant Physiol.*, **8**, 359-374.

Wang Z., Gerstein M. and Snyder M., 2009. RNA-Seq: a revolutionary tool for transcriptomics. *Nat. Rev. Genet.*, **10**, 57-63.

Waters D.L., Holton T.A., Ablett E.M., Lee L.S. and Henry R.J., 2005. cDNA microarray analysis of developing grape (*Vitis vinifera* cv. Shiraz) berry skin. *Funct. Integr. Genomics*, **5**, 40-58.

Yamaguchi-Shinozaki K., Kasuga M., Liu Q., Nakashima K., Sakuma Y., Abe H., Shinwari Z.K., Seki M. and Shinozaki K., 2002. *Biological Mechanisms of Drought Stress Response*. JIRCAS Working report 1-8.

Zamboni A., Di Carli M., Guzzo F., Stocchero M., Zenoni S., Ferrarini A., Tononi P., Toffali K., Desiderio A., Lilley K.S., Pe M.E., Benvenuto E., Delledonne M. and Pezzotti M., 2010. Identification of putative stage-specific grapevine berry biomarkers and omics data integration into networks. *Plant Physiol.*, **154**, 1439-1459.

Zenoni S., Ferrarini A., Giacomelli E., Xumerle L., Fasoli M., Malerba G., Bellin D., Pezzotti M. and Delledonne M., 2010. Characterization of transcriptional complexity during berry development in *Vitis vinifera* using RNA-Seq. *Plant Physiol.*, **152**, 1787-1795.

Zhang H.X. and Blumwald E., 2001. Transgenic salt-tolerant tomato plants accumulate salt in foliage but not in fruit. *Nature Biotechnol.*, **19**, 765-769.

Zheng J., Zhao J., Tao Y., Wang J., Liu Y., Fu J., Jin Y., Gao P., Zhang J., Bai Y. and Wang G., 2004. Isolation and analysis of water stress induced genes in maize seedlings by subtractive PCR and cDNA macroarray. *Plant Mol. Biol.*, **55**, 807-823.

Zottini M., Barizza E., Costa A., Formentin E., Ruberti C., Carimi F. and Lo Schiavo F., 2008. Agroinfiltration of grapevine leaves for fast transient assays of gene expression and for long-term production of stable transformed cells. *Plant Cell Rep.*, **27**, 845-853, doi: 10.1007/s00299-008-0510-4.

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