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’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
**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>
<thead>
<tr>
<th>Plant Species</th>
<th>Gene name</th>
<th>abiotic Stress</th>
<th>Technique used</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capsicum annum</td>
<td>Ca LEA1</td>
<td>Salt Stress, Water deficit, ABA</td>
<td>mRNA Differential display</td>
<td>(Park <em>et al.</em>, 2003)</td>
</tr>
<tr>
<td>Camellia sinensis</td>
<td>PR-5</td>
<td>Water deficit</td>
<td>mRNA Differential display</td>
<td>(Sharma et Kumar, 2005)</td>
</tr>
<tr>
<td>Pinus pinaster Ait.</td>
<td>PR-10</td>
<td>Water deficit</td>
<td>cDNA-AFLP</td>
<td>(Dubos et Plomion, 2001)</td>
</tr>
<tr>
<td>Triticum durum</td>
<td>AtGSK1</td>
<td>Salt Stress</td>
<td>cDNA-AFLP</td>
<td>(Chen <em>et al.</em>, 2003)</td>
</tr>
<tr>
<td>Zea mays</td>
<td>DREI/Rab17</td>
<td>Water deficit</td>
<td>SSH</td>
<td>(Zheng <em>et al.</em>, 2004)</td>
</tr>
<tr>
<td>Cicer arietinum</td>
<td>MIP</td>
<td>Water deficit</td>
<td>SSH</td>
<td>(Boominathan <em>et al.</em>, 2004)</td>
</tr>
<tr>
<td>Triticum turgidum</td>
<td>Alpha galactosidase</td>
<td>Salt stress</td>
<td>mRNA</td>
<td>(Hara <em>et al.</em>, 2008)</td>
</tr>
<tr>
<td>Tobacco</td>
<td>ASR1</td>
<td>Salt stress</td>
<td>Differential display</td>
<td>(Kalifa <em>et al.</em>, 2004)</td>
</tr>
<tr>
<td>Vitis vinifera</td>
<td>RD22</td>
<td>Salt stress</td>
<td>Candidate gene approach</td>
<td>(Hanana <em>et al.</em>, 2007; Jardak-Jamoussi <em>et al.</em>, 2014)</td>
</tr>
</tbody>
</table>

ABA = Abscisic Acid; AFLP = Amplified Fragment Length Polymorphism; SSH = Suppressive Subtractive Hybridization
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...
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.](image-url)
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).

<table>
<thead>
<tr>
<th>Clone ID</th>
<th>Genbank accession no.</th>
<th>CRIIBI Grape Genome annotation identifier</th>
<th>Homology/putative identity</th>
<th>6 h</th>
<th>6 h</th>
<th>24 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS-24H2-D1</td>
<td>GO238735</td>
<td>VIT_08s0007g08310</td>
<td>Alkaline alpha-galactosidase-seed imbibition protein (Vv-α-gal/SIP)</td>
<td>3.46 (0.84E-06)</td>
<td>2.92</td>
<td>6.78E-03</td>
<td>2.17</td>
</tr>
<tr>
<td>SS-24H3-F7.1*</td>
<td>GH717871</td>
<td>VIT_11s0016g05770</td>
<td>Alkaline α-galactosidase2</td>
<td>2.8 (1.75E-04)</td>
<td>2.17</td>
<td>2.10E-03</td>
<td>-</td>
</tr>
<tr>
<td>SS-24H3-F7.2*</td>
<td>GH717872</td>
<td>VIT_01s0150g00190</td>
<td>Transaldolase-like protein</td>
<td>2.8 (1.75E-04)</td>
<td>2.17</td>
<td>2.10E-03</td>
<td>-</td>
</tr>
<tr>
<td>SS-24H5-D12</td>
<td>GH717875</td>
<td>VIT_12s0028g03270</td>
<td>Ethylene-responsive transcription factor 4</td>
<td>2.32 (3.53E-05)</td>
<td>2.01</td>
<td>0.001925</td>
<td>-</td>
</tr>
<tr>
<td>SS-24H5-G6</td>
<td>GH717873</td>
<td>VIT_04s0023g01430</td>
<td>Zinc finger (C2H2 type) family protein</td>
<td>2.87 (1.60E-04)</td>
<td>2.58</td>
<td>1.45E-02</td>
<td>-</td>
</tr>
<tr>
<td>SS-6H1-D4</td>
<td>GH717874</td>
<td>VIT_03s0063g02110</td>
<td>Ubiquitin conjugating enzyme</td>
<td>2.45 (1.74E-05)</td>
<td>2.41</td>
<td>1.25E-02</td>
<td>-</td>
</tr>
<tr>
<td>SS-6H3-F3</td>
<td>GH717876</td>
<td>VIT_04s0023g00580</td>
<td>Unknown protein</td>
<td>2.98 (4.43E-04)</td>
<td>3.04</td>
<td>4.33E-03</td>
<td>-</td>
</tr>
</tbody>
</table>

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.
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 transcriptional 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
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., 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., 2009); 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.
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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