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Identification of differentially expressed genes in two grape varieties cultivated in semi-arid and temperate regions from West-Bank, Palestine

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ABSTRACT

Plants respond to stress conditions by altering genetic pathways. In this study, we aimed to identify and analyze differentially expressed genes in leaves of two grape varieties (genotypes) that were grown in Palestine either in a semi-arid region with a prolonged drought and high temperature stress or in a temperate region with moderate stress levels. In total, twelve transcripts with altered expression patterns, either by stress or genotype, were identified with the differential display RT-PCR (DDRT-PCR) technique and validated via quantitative real-time PCR (qRT-PCR). Eight transcripts represent genes that are down-regulated by stress in the leaves of at least one variety, among of which are members of the *DEAD-box RNA helicase*, *Haloacid Dehalogenase* (*HAD*) *hydrolase*, *kinesin-like*, and *mitochondrial Adenine nucleotide transporter* (*ANT*) gene families. Two genes encoding for members of the *GDSL Lipase/Esterase* and *Multiprotein Bridging Factor* (*MBF*) gene families were found to be up-regulated in stressed leaves. Two transcripts coding for a NAC-domain containing protein and a WD-repeat containing protein, respectively, were found to be non-responsive to those abiotic stresses but are differentially expressed in a genotype-dependent manner.

1. Introduction

Grape (*Vitis vinifera* L.) is one of the worldwide popular crops with an estimation of about 77 million tons of grape production in 2013 (FAOSTAT, 2015). Their cultivation is commercially remunerative as their major products, mainly wine, berries, seeds, and leaves are widely consumed and used in several industries (Iriti and Faoro, 2006; Monagas et al., 2006; Aguilar et al., 2016). In the Mediterranean Basin, grapevines are cultivated in the temperate climate zones and thus experience seasonal periods of drought (Medrano et al., 2003; Chaves et al., 2010). Unlike other crop plants, grapevines are relatively tolerant to moderate levels of drought. Moreover, despite the negative impacts of drought on the total yield, it has positive and desired effects on fruit and wine qualities (Medrano et al., 2003; Deluc et al., 2009; Van Leeuwen et al., 2009).

In Palestine, grape cultivation goes back to ancient historical periods (Gorr, 1966), and it is currently a major contributor to the Palestinian agricultural sector with an estimated annual production of about 80.000 tons (Harb et al., 2015). Several old and local as well as new and introduced varieties (genotypes) are cultivated, mainly in the Hebron governorate at the south of the West Bank. In the northern districts of that governorate, summers are long, hot, and rainless, whereas winters are short, cold, and rainy. The annual average precipitations are between 400 and 800 mm. In contrast, the southern districts of the governorate are considered as semi-arid regions with a lower annual precipitation rate (200–300 mm) (Harb et al., 2015). It is worth mentioning that grapevine cultivation in Hebron-West Bank relies solely on rainfall with no supplementary irrigation. This imposes significant stress on the plants, especially during the growth period in spring/summer, when water becomes scarce causing drought stress which is typically combined with heat stress due to higher summer temperatures. Accordingly, especially in the southern parts of West

Abbreviations: ABA, abscisic acid; ANT, adenine nucleotide transporter; cDNA, DNA complementary to RNA; DDRT-PCR, differential display reverse transcription-polymerase chain reaction.; DNase, deoxyribonuclease; dNTP, deoxyribonucleoside triphosphate; DTT, dithiothreitol; DXS, 1-Deoxy-D Xylulose-5-Phosphate synthase; G6PDH, Glucose-6-Phosphate 1-Dehydrogenase; HAD, Haloacid Dehalogenase; HCF106, High Chlorophyll Fluorescence 106; KAC, Kinesin like protein for actin based chloroplast movement; MBF1a, Multiprotein Bridging Factor 1a; MEP, Methyl-Erythritol Phosphate; MLA *R*, mildew resistance (*R*) locus A; NAC, Petunia *NAM* (no apical meristem) and Arabidopsis *ATAF1*, 2 and *CUC2*; NPQ, non-photochemical quenching; qRT-PCR, quantitative real-time PCR; ROS, reactive oxygen species; SA, semi-arid; SDS, sodium dodecyl sulfate; SOG1, Suppressor of Gamma response 1; SOQ1, Suppressor of Quenching 1; SPPL, Signal Peptide Peptidase-Like; T, temperate; THF1, Thylakoid Formation 1; TOGR, Thermotolerant Growth Required 1

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Bank, the grape varieties must have developed adaptation mechanisms to cope with these harsh conditions. Studying such mechanisms was and still is a major topic in plant sciences.

Drought is considered as a major limiting factor for plant growth, performance, and productivity, causing serious agricultural yield losses worldwide. Plants' tolerance to abiotic stressors (i.e. drought) is known to be triggered by complex multicomponent signaling pathways, which restore cellular homeostasis and promote survival and adaptation. Stress-induced responses involve the differential expression of large sets of genes that are essential to drive such changes (Huang et al., 2008; Harb et al., 2010). Drought is known to trigger the production of the plant hormone abscisic acid (ABA) (Finkelstein et al., 2002) and numerous studies revealed that ABA and its corresponding signaling pathway forms a major part of the drought response regulatory network in plants (Zhu, 2002; Davies et al., 2005; Huang et al., 2008). Several ABA-related transcription factors (TFs) were reported to operate in drought signaling pathways (Tuteja, 2007; Golldack et al., 2014; Savoi et al., 2017). These TFs are identified to modulate the expression of downstream ABA-responsive genes and this modulation eventually leads to several cellular and physiological responses such as increasing levels of cytoplasmic organic osmolytes (Munns and Tester, 2008) and stomatal closure (Zhu, 2002; Davies et al., 2005). Moreover, organ and/or tissue specificity with regard to ABA signaling and responses is known in plants including grapes (Finkelstein, 2013; Rattanakon et al., 2016). Nevertheless, ABA-independent pathways, including gibberellic acid (GA)-, jasmonate (JA)-, reactive oxygen species (ROS)-, and lipid-dependent pathways, mediate drought-induced responses with growing evidences for cross-talks between them (reviewed in Kuromori et al., 2013; Golldack et al., 2014).

Under field conditions, however, plants are often concurrently exposed to several abiotic/biotic stress combinations (i.e. drought and heat stresses). Despite the fact that various components are shared in the signaling pathways for different stresses (reviewed in Pandey et al., 2015), recent studies indicated that plant responses to stress combinations varies significantly at molecular and physiological levels and cannot be deduced from the responses to specific stresses applied under controlled conditions (reviwed in Mittler, 2006; Suzuki et al., 2014). In addition, plant responses to a simultaneous occurrence of different stresses tend to be highly complex as it results from different, and sometimes opposing, signaling pathways that may interact and/or inhibit each other (Mittler, 2006; Suzuki et al., 2014). Thus, it is necessary to study plant tolerance and adaptation to stress combinations under conditions mimicking field environment or real field conditions.

In this study, we aimed to investigate and identify differentially expressed genes that potentially act in grapevine plants adapted to drought and high temperature stresses under field conditions. For this, two local grapevine varieties, namely “Beituni” and “Shami”, were selected that are widely cultivated in Palestine. This study is a further step toward not only a better characterization of local Palestinian grape varieties, in particular their adaptation to the local harsh environmental conditions, but also to better understand adaptation mechanism in grapes under combined stress conditions.

2. Material & Methods

2.1. Plant material

Grapevine leaves from “Beituni” and “Shami” varieties were collected from two different geographic regions of the West Bank-Palestine. The first location is Al-Dahria, which is 655 m above sea level and considered as a semi-arid region (average summer temperature = 25 °C; average annual rainfall rate = 255 mm; and potential monthly evapo-transpiration of 101.4 mm for the June–September period). The second location is Beit Ommar, which is 987 m above sea level and considered as a temperate region (average summer temperature = 21 °C; average annual rainfall rate = 500 mm, and potential monthly evapo-transpiration of 100.6 mm for the June–September period). The selection of these locations aimed to assess the influence of severe abiotic stresses, in particular drought and high temperature, in the semi-arid region compared to the moderate abiotic stresses in the temperate region. The collection time of leaves was during the main flush of vegetative growth (June 2013). Healthy leaves without any apparent infection symptoms were harvested and directly snap-frozen in liquid nitrogen and kept after that at − 80 °C until subsequent analyses.

2.2. RNA extraction and cDNA synthesis

Grape leaves were ground to fine powder under liquid nitrogen. 300 mg per sample were taken for total RNA extraction according to Chang et al. (1993) with slight modifications, namely the pellet was dissolved in SSTE buffer (1 M NaCl, 0.5% SDS, 10 mM Tris-HCl (pH 8), 1 mM EDTA (pH 8)) before the final separation using chloroform:isoamylcohol (24:1) solution. RNA quality and quantity were assessed by gel-electrophoresis and NanoDrop spectrophotometer (Thermo Fischer Scientific), respectively. Genomic DNA contaminants were digested by DNaseI (NEB) for 1 h at 37 °C, and 6 µg of DNA-digested RNA were used further for cDNA synthesis. The first strand synthesis was performed in a total volume of 20 µL using Superscript III RT (Thermo Fischer Scientific). The reaction setup was as follows: 6 µg of RNA was mixed with 1 µL of 100 µM for one of the base-anchored primers (Table S1), and 1 µL of dNTPs (10 mM each). The mixture was incubated at 65 °C for 5 min and then transferred onto ice for 3 min. After that 4 µL of 5 × first strand buffer, 1 µL 0.1 M DTT, 1 µL of Superscript III RT (200 units), and water were added to a final volume of 20 µL. The reaction was incubated at 50 °C for 60 min followed by 15 min at 70 °C.

2.3. Differential display RT-PCR (DDRT-PCR)

Second strand synthesis and PCR amplification was performed in a 20 µL reaction mixture, using 2 µL of RT mix from the first strand cDNA. Each reaction mixture contains 2 µL of 10 × PCR buffer, 0.5 µL dNTPs (10 mM each), 2 µL each of one anchored primer (Table S1), one of the arbitrary primers (Table S1), and 0.25 µL Taq polymerase (5 U/µL, Genaxxon bioscience). The PCR reactions were as follows: 95 °C for 30 s followed by primer annealing at 40 °C for 2 min, extension at 72 °C for 30 s for 28 cycles followed by final extension at 72 °C for 5 min.

The DDRT-PCR products were loaded onto 2% agarose gels containing ethidium bromide (0.25 µg/mL) and separated by electrophoresis at 100 V. Amplification products were visualized by UV light and the product sizes were determined by comparison against the GeneRuler 100 bp plus DNA marker (Thermo Fisher Scientific). PCR products representing transcripts with differential expression patterns between either the two genotypes or the two geographical regions were excised from the gels and eluted using NucleoSpin® Gel and PCR Clean-up kit (Macherey-Nagel). The eluted bands were re-amplified and re-eluted from the agarose gel, cloned into pJET1.2/blunt end cloning vector (Thermo Fisher Scientific), and transformed into DH5α *E.coli* competent cells that were selected on LB agar plates supplemented with 100 µg/mL ampicillin. Bacterial colonies harboring insert-containing plasmids were picked for propagation and plasmid purification for sequencing using NucleoSpin® Plasmid EasyPure kit (Macherey-Nagel).

2.4. Analysis of obtained ESTs

The obtained cDNA nucleotide sequences were analyzed by homology searches using BLASTN and BLASTX (Altschul et al., 1990) against the *V. vinifera* genome sequences deposited in the “EnsemblPlant” (http://plants.ensembl.org/index.html) and the NCBI databases (Altschul et al., 1990). BLASTX against the *Arabidopsis thaliana* database (https://www.arabidopsis.org/) was performed to search for