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Global transcriptome analysis of heat stress response of grape variety 'Fantasy Seedless' under different irrigation regimens

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Summary

Grapevine (*Vitis vinifera* L.), a commercially important fruit crop worldwide, faces several challenging conditions during its growth cycle. Among many abiotic stresses, heat and moisture stresses have major impact on grapevine productivity and fruit quality. Transcriptome analysis of heat stress response of grape variety 'Fantasy Seedless' grown under different irrigation regimens identified large number of differentially expressed genes. Genes belonging to chaperone mediated protein folding and cell-wall modification pathways were found to play a significant role in plant response to heat as well as moisture stress. Subsurface irrigation helped minimize the adverse effects of stress through modulation of genes involved in cell homeostasis. The study has given critical insight into grapevine response to heat stress arising due to aberrant weather conditions.

K e y w o r d s : grape; heat stress response; protein folding; cell homeostasis.

Introduction

Grape (*Vitis vinifera* L.) is one of the most important fruit crop globally. It is cultivated over an area of 7,157,658 ha (FAOSTAT 2008) under different climatic conditions.

During its growth cycle, grapevine experiences many biotic and abiotic stresses. Heat stress is one of the major abiotic stresses affecting grape production and quality. Heat stress is defined as the occurrence of temperature above the optimum. In grape, temperatures above 35 °C results in reduced photosynthesis in leaves (Kriedemann 1968). The breakdown of cellular organization due to high temperatures leads to cell injury or even cell death (Wahid *et al.* 2007). Injuries due to heat includes protein denaturation and aggregation, enzyme inactivatin, loss of membrane integrity resulting in generation of reactive oxygen species and other toxic compounds and causing metabolic imbalance. In many grape growing regions, the day temperature rises above 40 °C which affects grapevine phenology, resulting in altered growth pattern and affecting grape quality (Greer and Weedon 2013, Abeysinghe *et al.* 2019). In grape, several researchers have studied the physiological changes like net photosynthesis, hormone changes, cell signaling etc. (Wang *et al.* 2009, Luo *et al.* 2011, Greer and Weedon 2013), grapevine performance (Greer and Weedon 2013) and quality (Mori *et al.* 2007) in response to heat stress. In recent years, transcriptome analysis has given much insight into molecular mechanism of heat stress response in grape. Liu *et al.* (2012) reported association of heat stress and recovery with multiple processes and mechanisms including stress-related genes, transcription factors, and metabolism. Transcriptome analysis of berries under different high temperatures revealed induction of HSP proteins, which probably facilitate berry ripening through stabilization of protein functions and transmembrane transporter density (Carbonell-Bejerano *et al.* 2013). Rienth *et al.* (2014) observed differences in heat stress responsive pathways according to day or night treatment especially for genes involved in acidity and phenylpropanoid metabolism.

The high rate of evaporation under elevated ambient temperature conditions leads to reduced soil moisture and subsequent moisture stress to the grapevine. Grapevine is considered moderately tolerant to moisture stress and is well adapted to semi-arid climate (Chaves *et al.* 2010). The water deficit is often used to enhance aroma and improve berry composition of wine grapes. However, in table grapes, deficit irrigation at critical stages of growth adversely affects berry size and quality as well as yield (Zúñiga-Espinoza *et al.* 2015, Permanhani *et al.* 2016). The extent of adverse effect depends on soil type, time of stress and climate during growing season besides cultivar and rootstock type (Cardone *et al.* 2019). Physiological response of grapevine to water deficit stress has been studied in detail and comprehensively reviewed by Chaves *et al.* (2010). Transcriptome analysis of water stress revealed cultivar specific modulation of genes in response to water stress (Catacchio *et al.* 2019) and consisting of a multi-step component system involving several genes regulating various pathways (Cramer *et al.* 2007, Haider *et al.* 2017). Under field conditions, a variety of abiotic stresses like heat, water, salinity and oxidative stress occur simultaneously. Plants use a diversity of mechanisms and combinations of mechanisms to tolerate each of these stresses (Roy *et al.* 2011). The combined effect of heat and drought is higher than the individual stress and elicit different response as compared to the individual stress (Grigorova *et al.* 2011). In grape, several researchers have studied grapevine response to drought, salinity and co-occurring stresses (Cramer *et al.* 2007, Chaves *et al.* 2010, Cramer 2010, Rocheta *et al.* 2014) at transcript level. Increased temperatures

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resulting in higher reference evapotranspiration values and more frequent years with low rainfall will continue to induce more intense and frequent drought conditions for vineyards around the world (van Leeuwen *et al.* 2019). Therefore, understanding of grapevine response to different climate variables is critical for developing adaptation strategies as well as identifying or developing cultivars with phenotypic plasticity. In this paper we report transcriptome analysis of table grape variety 'Fantasy Seedless' grown under different irrigation regimes and experiencing heat stress due to elevated ambient temperature.

Material and Methods

Plant material: The experiment was conducted on grape variety Fantasy Seedless grafted on rootstock 110R and raised on Y-trellis system at vineyards of ICAR-NRC for Grapes, Pune during the month of May. During the experiment, the vines were in active shoot growth stage. A pan evaporation based crop stage wise irrigation schedule was used to irrigate the vines. Four irrigation schedule treatments were being implemented in this plot. Vines in treatment 1 were receiving irrigation through drip as recommended for grape variety 'Thompson Seedless' (designated as R), treatment 2 vines received 80 % of recommended irrigation through drip (R80 %), treatment 3 received 50 % of recommended irrigation through drip (R50 %), and treatment 4 also received 50 % of recommended irrigation but through sub-surface irrigation (RS50 %). The experimental vines received irrigation as per this schedule three days before the sample collection.

Sampling under high temperature conditions in the field: The maximum day temperature on the day of sampling reached 38 °C at 11.30 AM and remained between 38-40 °C till 4.00 PM. The hourly temperature data is given in suppl. Fig. 1. The first set of samples was taken between 7.45 AM to 8.00 AM when the air temperature was 22-24 °C and these samples were considered control samples. The second set of samples was collected between 3.45-4.00 PM, after the vines were exposed to extreme temperatures for at least four hours and these samples were considered heat stressed samples. The shoot tips (containing unopened and first opened leaves) were collected from three different vines, snap frozen in liquid nitrogen and stored at -80 °C till use. Same vines were used for sampling at both the times.

Physiological observations: Physiological observations like transpiration rate, assimilation rate and leaf water potential were measured using Infra Red Gas Analyzer GFS300 (Waltz, Germany). The data were analyzed using SAS statistical software (SAS Inc. USA).

RNA extraction: RNA was extracted from 70-100 mg of pooled sample using the Spectrum Plant RNA extraction kit (Sigma-Aldrich, USA) as per the manufacturer's instructions. On-column DNase digestion was performed before RNA elution from column. The quality of extracted RNA was assessed using the RNA 6000 Nano Kit with an Agilent 2100 Bioanalyzer (Agilent Technologies, UK) and only the high quality RNA samples with a RIN value of more than 8 were used for library preparation.

Library preparation, RNA sequencing and data processing: Poly(A) mRNA was prepared from approximately 2.5-µg of high quality total RNA for each sample. A non-directional Illumina RNA-seq library was prepared using the TruSeq RNA Sample Prep Kit v2 (Illumina, USA). High Sensitivity DNA Kit (Agilent, UK) was used to check the quality of library. The libraries were sequenced using an Illumina HiSeq 2000 sequencer (Illumina, USA) and 101-bp paired-end sequences were generated. The sequencing services were performed by AgriGenome Labs Pvt Ltd. Kochi. The Galaxy web platform (Afgan *et al.* 2018) available at https://usegalaxy.org was used for pre-processing, alignment and count estimation. Cutadapt was used to remove low-quality reads (< 50 bases, minimum quality 20), putative PCR duplicate reads and Illumina TruSeq adapter sequences. The pre-processed reads were aligned to the reference *Vitis vinifera* genome and gene models downloaded from Ensembl database (http://ftp.ebi.ac.uk/ensemblgenomes/pub/release-47/plants/gtf/vitis_vinifera/). The alignment was performed using HISAT2 with default parameters. Only the uniquely mapped reads were used for further analysis. The aligned reads were used for estimating the expression counts of the genes and transcripts using FeatureCounts.

Differential expression data analysis: Count data were used for differential expression analysis using R Bioconductor package NOISeq following NOISeq-sim method recommended for no replicate dataset (Tarazona *et al.* 2015). The list of DEGs was filtered based on the criteria Log_2(fold change) ≥ 1.5 and probability ≥ 0.95 (FDR0.05) to select the most significant DEGs. Functional categorization of the differentially expressed transcripts and gene enrichment analysis was performed using Blast2GO. Web-based analysis software Morpheus (https://software.broadinstitute.org/morpheus/) was used for cluster analysis and generating heat map. Software MapMan (Thimm *et al.* 2004) was used for pathway using GrapeGen 12Xv1 MapMan version (Carbonell-Bejerano *et al.* 2013).

Results and Discussion

Imposition of heat stress: The optimum temperature for grapevine growth is between 25 and 35 °C (Mullins *et al.* 1992). A temperature rise of 5 °C above the optimal induces stress for the vines. Many workers have used a temperature of 38-45 °C to study impact of heat stress on grapevine (Liu *et al.* 2012, Carvalho *et al.* 2015, Lecourieux *et al.* 2017). In our experiment also grapevines experienced an ambient temperature of 40 °C for at least four hours (suppl. Fig. 1), a duration sufficient to elicit stress response at molecular level.

Physiological observations: The data on assimilation rate, transpiration rate and leaf water potential during the experiment is given in Fig. 1. Assimilation rate and leaf water potential varied significantly among treatments. Transpiration rate did not vary significantly among

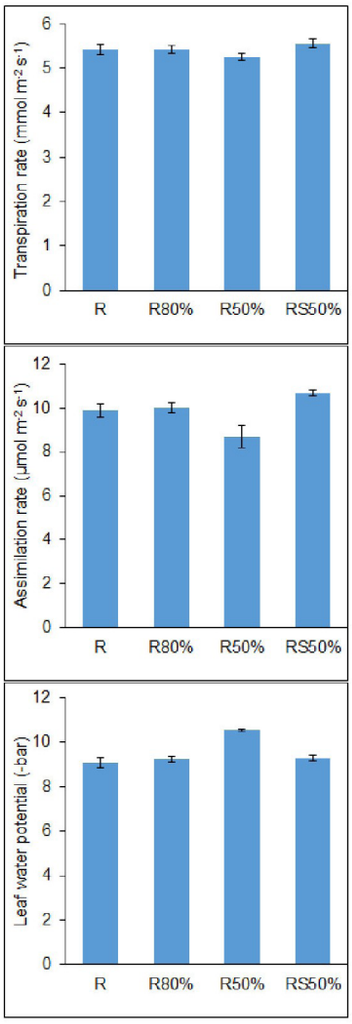

Fig. 1: Physiological data of experimental vines of 'Fantasy Seedless'. Data showed that the vines receiving 50 % of recommended irrigation (R50%) were experiencing stress.

treatments, however grapevines receiving 50 % irrigation had lower rate of assimilation and higher leaf water potential. (suppl. Tab. 1). In grape, reduced photosynthetic activity and CO_2 assimilation rate under drought stress have been reported by several workers. Our results indicated that R50% resulted in physiological stress, however the subsurface method mitigated the adverse effect of deficit irrigation as physiological activity of these vines were at par with vines receiving full irrigation. Subsurface irrigation has been demonstrated to increase yield and improve water use efficiency (Sharma and Upadhyay 2011, Pisciotta *et al.* 2018, Ma *et al.* 2020).

Differentially expressed genes

Heat stressed responsive genes: A total of 4988 genes were differentially expressed in response to heat stress across all irrigation treatments, however only 1234 genes were significant as per the selection criteria (Log_2 (fold change) ≥ 1.5 or ≤ -1.5) (suppl. Tab. 2) and were considered for further analysis. The number of significant differentially expressed genes in heat stressed vines receiving recommended irrigation (RT) was 713 (271 down-regulated, 442 up-regulated), DEGs in vines receiving 80 % of recommended irrigation (R80%T) was 764 (319 down- and 445 up-regulated), 647 genes (257 down- and 390 up-regulated) were differentially expressed in heat stressed vines receiving 50 % of recommended irrigation (R50%T), whereas 508 genes (228 down- and 280 up-regulated) were significant in heat stressed vines receiving 50 % irrigation through sub-surface irrigation (RS50%T). In all the analyses, the number of up-regulated genes was higher than the down-regulated genes (Fig. 2). 263 DEGs were common

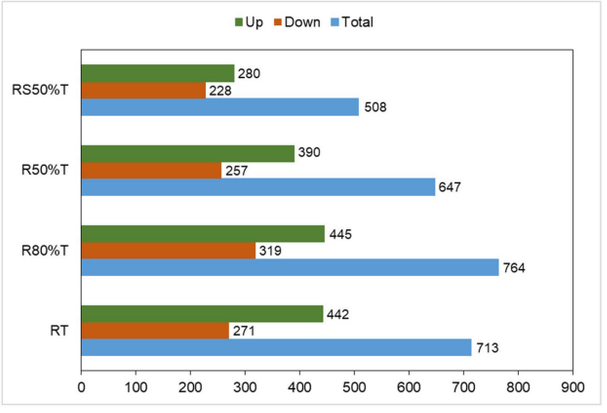

Fig. 2: Details of differentially expressed genes in different heat stress samples. The number of upregulated genes was more than the downregulated genes in all the treatments.

among all the analyses. The number of unique genes was maximum for RT (173 genes) followed by R80%T (158) and R50%T (133) and the least number of unique DEGs was detected in Rs50%T (72; Fig. 3).

Moisture stress responsive genes: We also compared data of control vines receiving sub-optimal levels of irrigation (80 % and 50 %) with recommended level of irrigation to identify the moisture stress responsive genes. The number of DEGs in response to moisture stress was much less as compared to heat stress. 320 genes were differentially expressed and only 42 were significant based on selection criteria (suppl. Tab. 3a). The number of significant DEGs was 9 (3 up- and 6 down-regulated), 36 (1 down- and 35 up-regulated) and 32 (2 down- and 30 up-regulated) in vines receiving respectively 80 % (R80%C), 50 % (R50%C), 50 % (through subsurface, RS50%C), of recommended ir-

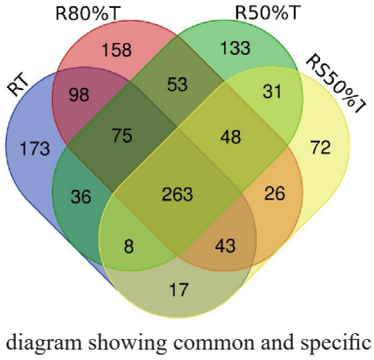

Fig. 3: Venn diagram showing common and specific DEGs under heat stress in different irrigation treatments. Large number of treatment specific DEGs were observed under heat stress.

rigation level. Only four genes were common among three irrigation treatment. However, 24 genes were common between vines receiving 50 % irrigation through drip and subsurface irrigation (Fig. 4). 96 genes were differentially expressed in vines receiving 50 % irrigation through subsurface when compared with 50 % irrigation through drip. However, as per selection criteria (Log_2 (fold change) ≥ 1.5 or ≤ -1.5), only 9 genes were significant (suppl. Tab. 3b).

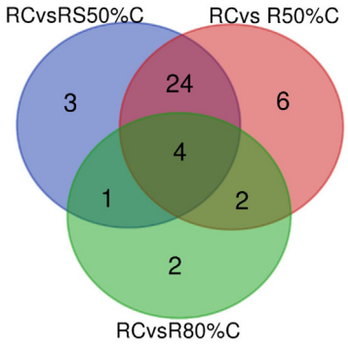

Fig. 4: Venn diagram of moisture stress responsive DEGs. Only a few genes were common among all the treatments, however, several genes were common between vines receiving 50 % irrigation through drip and subsurface irrigation.

Effect of irrigation level on heat stress response: We also performed pairwise comparison between heat stressed vines with 100 % and heat stressed vines receiving deficit irrigation. 1154 genes were found significant, however, only 86 genes were significant as per the selection criteria (suppl. Tab. 4a). 21 genes (all down-regulated) were differentially expressed in R80%T when compared with RT samples. The number of DEGs in R50%T increased to 54 (11 up- and 44 down-regulated) *vis a vis* RT. However, the number of DEGs decreased to 39 in RS50%T (17 up- and 22 down-regulated) when compared with RT.

Forty genes were significantly expressed differentially in vine receiving 50 % of recommended irrigation through subsurface as compared to vine receiving 50 % irrigation through drip (suppl. Tab. 4b). Of the 40 DEGs in vines receiving irrigation through subsurface, 32 were up-regulated. Among up-regulated genes, 11 were small HSPs involved in chaperone mediated protein folding. Some of the sHSPs were uniquley up-regulated in subsurface irrigation treatment. Irrigation through subsurface results in improved water use efficiency while maintaining the yield of quality grapes (Sharma *et al.* 2005). Our results suggested that subsurface irrigation helps minimize adverse effects of stress through modulation of genes involved in cell homeostasis.

Functional annotation of DEGs: The blast2go analysis categorized heat responsive DEGs into three classes viz. biological processes, molecular function and cellular components. The top 20 GO term in each category are depicted in suppl. Fig. 2a. At level three of gene ontology, primary metabolic process, organic substance metabolic process, nitrogen compound metabolic process, cellular metabolic process, and biosynthetic process were the top five GO terms related to biological processes. Though not among the top five terms, response to stress and response to abiotic stimulus were the other important BP GO terms. Among molecular function, heterocyclic compound binding, organic cyclic compound binding, transferase activity, small molecule binding and hydrolase activity were the top GO terms. Whereas, intracellular organelle, organelle, cytoplasm, membrane and cell periphery were the top five level three cellular component GO terms.

The top 20 GO term in each category for moisture stress responsive genes are depicted in suppl. Fig. 2b. At level three of gene ontology, response to stress, organic substance metabolic process, and primary metabolic process were the top three GO terms related to biological processes. Among molecular function, organic cyclic compound binding, heterocyclic compound binding and small molecule binding were the top GO terms. Cytoplasm, intracellular organelle and organelle were the top level 3 GO terms related to cellular components.

In case of combined heat and moisture stress response the top three biological process GO terms were response to stress, primary metabolic process and organic substance metabolic process binding, molecular function terms were heterocyclic compound binding, organic cyclic compound binding and transferase activity whereas cellular component GO terms were cytoplasm, organelle and intracellular organelle (suppl. Fig. 2c).

GO Term enrichment analysis: Fisher's exact test was used to identify the enriched GO terms in DEGs. A total of 10 GO terms were enriched (Fig. 5a) in response to heat stress. Among these, five GO terms including response to stress (GO:0006950) and response to abiotic stimulus (GO:0009628) were over-represented whereas the remaining

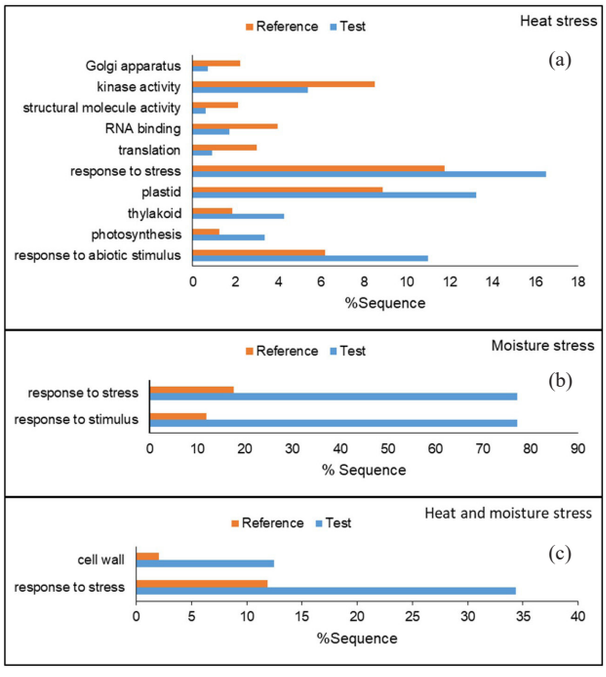

Fig. 5: Gene enrichment analysis of significant DEGs. The GO term "response to stress" was enriched under all stress conditions.

5 terms were under-represented. In response to moisture stress, two GO terms; response to stimulus and response to stress were enriched and both the terms were over-represented (Fig. 5b). Similarly 86 DEGs detected for combined heat and moisture stress, had overrepresentation of two GO terms viz. cell wall and response to stress (Fig. 5c).

Pathway analysis: In MapMan analysis, heat stress responsive differentially expressed genes represented different pathways like primary metabolism (252), signaling (109), regulation (99), response to stimulus (53), transport (65) etc. A large number of differentially expressed genes (237) have unknown function (suppl. Tab. 5). In majority of the pathways, the number of up-regulated genes was more than the down-regulated genes (Fig. 6). Pathway related to protein metabolism and modification, a part of primary metabolism was significantly affected by heat stress (Table). Besides this, pathway related to temperature stress response and cellular component organization and biogenesis were also significant. Majority (29/42) of the moisture stress responsive genes belonged to protein metabolism and modification pathway involved in HSP mediated protein folding and a few genes were related to stress response pathway and signaling (suppl. Tab. 6).

Protein metabolism and modification pathway, the significant pathway contained sixty four genes involved in chaperone-mediated protein folding and 28 genes involved in proteolysis which were modulated in heat treated samples (suppl. Tab. 5). Chaperones are major components of cell homeostasis under optimal as well as stress conditions of growth (Wang *et al.* 2004). Under optimal growth condition, chaperones are responsible for protein folding, assembly, translocation and degradation in a broad array of normal cellular processes whereas under stress condition they function in the stabilization of proteins and membranes, and can assist in protein refolding. A small increase in temperature may result in protein unfolding, entanglement, and unspecific aggregation (Richter *et al.* 2010). Under heat stress, the first fast expression phase corresponds to processes that rapidly counteract the consequences of heat shock, whereas the later phases represent adaptation or recovery processes. Small HSPs with a molecular weight between 15-42 kDa are classified as HSP20 proteins. HSP20 are considered to be the first line of defense under stress. Chaperone HSPs interact with partially folded target proteins to prevent their aggregation under stress (Mchaourab *et al.* 2009) and are efficient in preventing irreversible aggregation processes. Upregu-

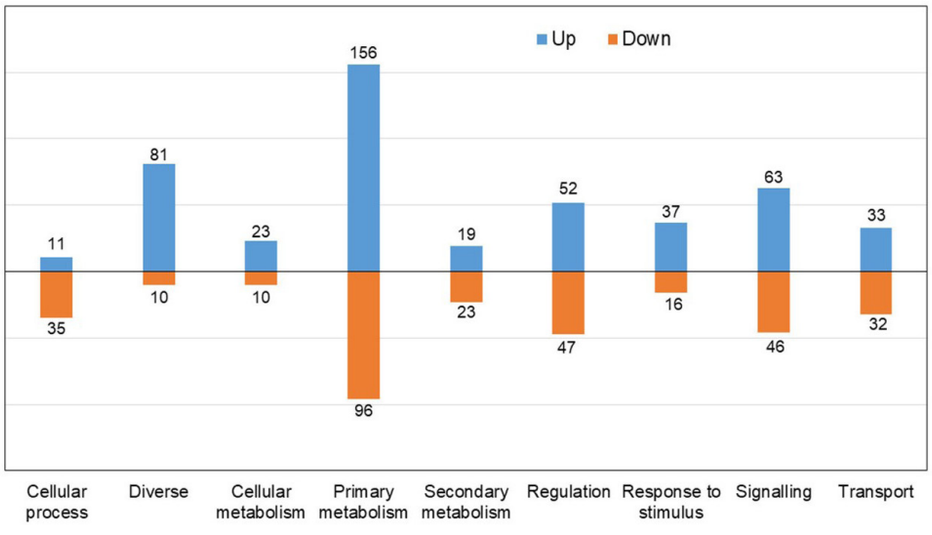

Fig. 6: Grouping of DEGs in different pathways. The number of upregulated genes was more than the downregulated genes in most of the pathways.

Table

Significant pathways represented by heat stress responsive differentially expressed genes

Bin	Name	Elements	*p*-value
4.2	Metabolism. Primary metabolism	249	0.0035
4.2.10	Metabolism. Primary metabolism. Protein metabolism and modification	96	6.344E-14
4.2.10.1	Metabolism. Primary metabolism. Protein metabolism and modification. Protein folding	62	0.0
4.2.10.1.1	Metabolism. Primary metabolism. Protein metabolism and modification. Protein folding. Chaperone-mediated protein folding	59	0.0
4.2.10.1.1.2	Metabolism. Primary metabolism. Protein metabolism and modification. Protein folding. Chaperone-mediated protein folding. HSP-mediated protein folding	55	0.0
6.2.1.8	Response to stimulus. Stress response. Abiotic stress response. Temperature stress response	15	0.002
1.2	Cellular process. Cellular component organization and biogenesis	39	0.002

lation of small HSPs in response to abiotic stress is well documented by several workers. In grapevine, 48 HSP20 proteins have been identified (Ji *et al.* 2019). In our analysis 38 HSP20s were up-regulated in response to heat stress with varied level of expression across four treatments. 9 HSP20s were common among four treatments. The level of upregulation was the maximum in vines receiving recommended level of irrigation as compared to the vines receiving 80 % and 50 % irrigation (Fig 7A). The expression of 21 of these HSP20s was modulated in response to moisture stress also (suppl. Tab. 3). The significant modulation of HSP20s are in accordance with earlier reports by Liu *et al.* (2012) and Carvalho *et al.* (2015) who reported upregulation of HSP20s during heat stress in grapevine. Haider *et al.* (2017) observed upregulation of HSPs in response to drought stress in leaves of grape variety 'Summer Black'. The role of some of the HSP20s with significant modulation in response to both heat and moisture stress needs to be explored for their use as candidate genes for multiple tolerance.

The expression of HSPs and many other stress responsive genes are regulated by Heat shock factors (Hsfs) (Schramm *et al.* 2006, Scharf *et al.* 2012). In our study, three HsfA and 2 HsfB were differentially expressed. Class A Hsf are reported to positively regulate the expression of heat response genes (Scharf *et al.* 2012), whereas Class B Hsfs have been shown to interact with class A-Hsf in regulating the shut-off of the heat shock response in *Arabidopsis* (Kumar *et al.* 2009). Expression of three HsfA genes was upregulated in response to heat stress under varied irrigation regimens, though the extent of upregulation varied for three genes. The expression of HsfA6B_2 increased 30 fold (log2(fold change) = 4.89) in heat stressed vines receiving full irrigation, whereas fold increase was only 8, 4.5, 5.0 respectively in vines receiving 80 %, 50 % levels of irrigation and 50 % through sub-surface irrigation. Under moisture stress also, expression of HsfA6B_2 increased significantly in vine receiving 50 % level of irrigation, suggesting the role of this transcription factor in multiple stresses. On the other hand, expression of HsfA6B_4 increased significantly only in heat stressed vines receiving full irrigation. The expression of class B Hsfs, HsfB2b and HsfB2a was downregulated in heat stressed plants. The extent of downregulation was higher in vines receiving deficit irrigation (Fig 7B). In *Arabidopsis*, Huang *et al.* (2016) demonstrated the positive regulatory role of HsfA6b in ABA-mediated salt and drought resistance and acquisition of thermotolerance. Hu *et al.* (2016) reported upregulation of HsfA6b in *Vitis pseudoreticulata* in response to different stresses including heat and suggested its role in basal thermotolerance *via* hormone dependent signaling pathways. In our analysis also, a large number (32 genes) of hormone signaling genes were modulated. Among these genes, all the 5 significant ABA signaling genes were up-regulated, most genes (9/10) for ethylene signaling were up-regulated, whereas most of auxin signaling genes (8/12) were down-regulated. The detailed analysis is needed to understand the interaction of Hsfs and hormone signaling genes and their role in heat and moisture stress response. Besides hormone signaling, we also detected

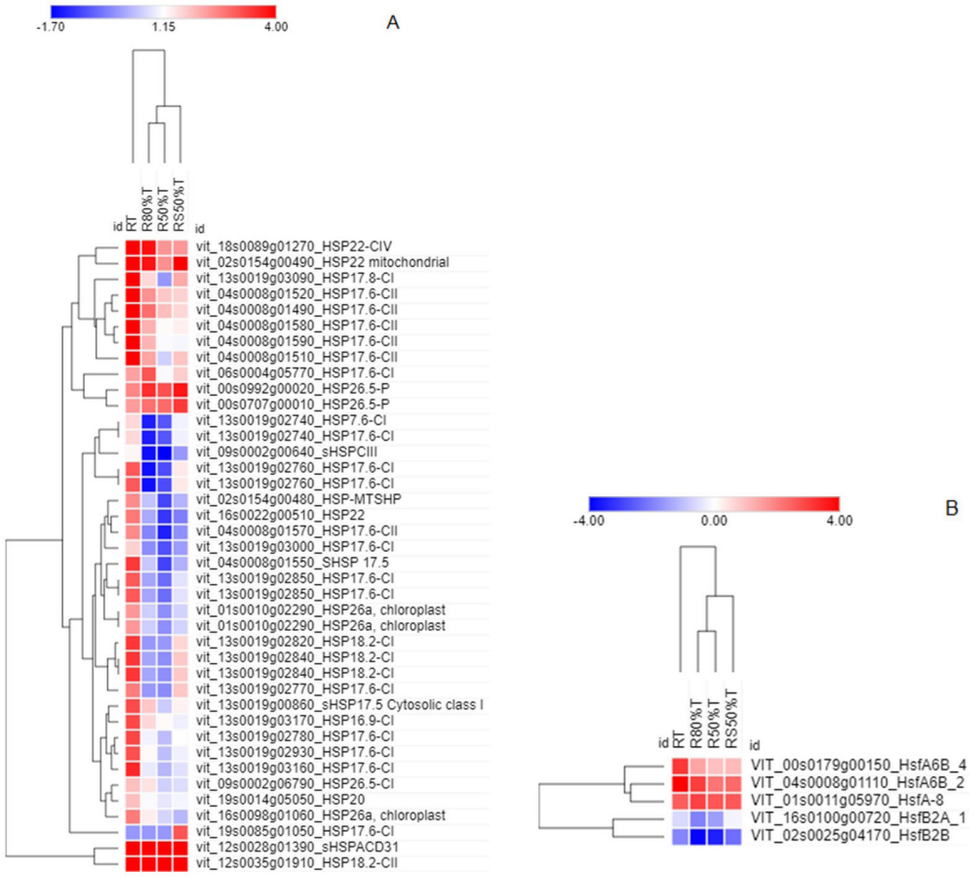

Fig. 7: Expression profile of HSP20 proteins (**A**) and Hsfs (**B**) across four treatments. The level of upregulation of HSP20 proteins in heat stress samples was higher in vines receiving recommended irrigation.

77 genes for other signaling pathways like Calcium sensors and signaling (8 genes), Circadian clock signaling (8 genes), G-protein signaling (9 genes) and Protein kinase (31 genes). Modulation of a large number of protein kinase gene indicates their role in heat stress response and requires detailed analysis. Pathway analysis also identified Cellular component organization and biogenesis as significant pathway in response to heat stress under different irrigation regimes. 23 genes involved in cell wall metabolism were modulated in response to heat stress. The genes involved in cell wall synthesis were down-regulated, whereas most of cell wall modification genes like Xyloglucan endotransglucosylase/hydrolase (XET/XTH) genes and pectin methylesterase (PMEs) genes were up-regulated. Cell wall restructuring is considered as an important component of plant response to heat stress in order to sustain cell function and growth. Cell wall-related proteins are involved in modulation of cell wall extensibility, which mediates cell enlargement and expansion (Le Gall *et al.* 2015). XET/XTHs involved in the modification of cell wall structure by cleaving and re-joining xyloglucan molecules in primary plant cell walls, are major proteins involved in response to several abiotic stresses. Lecourieux *et al.* (2017) observed an increased level of XETs transcripts in green berries subjected to local heat treatment. In leaves also, an increase in cell wall elasticity contributes to the maintenance of cell turgor under stress conditions (Saito and Terashima 2004). Similarly, PMEs control cell wall composition by assembly and disassembly of pectin network especially in guard cell walls for regulating cell wall plasticity under stress (Wu *et al.* 2018, Huang *et al.* 2017). We observed varied response of XET/XTH and PME genes in grapevines receiving full and deficit irrigation (suppl. Fig. 3), thus highlighting the importance of these cell wall modifying genes in maintaining cell turgor and metabolism during stress conditions.

Besides these significant pathways, we observed modulation of large number (70 nos.) of Pentatricopeptide (PPR) repeat-containing protein. PPR domain-containing proteins have an RNA binding domain and are mainly involved in the regulation of plant growth and development. However, several recent reports have shown their vital role in plant response to several biotic and abiotic stresses in different crops (Xing *et al*. 2018, Jiang *et al.* 2015). Several PPR proteins are involved in post-transcriptional and post-translational processes in response to biotic and abiotic stresses (Laluk *et al.* 2011). We observed upregulation of 69 out of 70 PPR proteins, though the expression pattern varied among different treatments (suppl. Fig. 3). The increased expression of PPR genes may depend on the expression of their target genes. These results are in accordance with He *et al.* (2019) who reported upregulation of PPR transcripts in maize leaves in response to heat stress. The upregulation of PPR in response to drought and other biotic and abiotic stresses has also been reported (Chen *et al.* 2018). However, our results are contrary to reports by Zhang *et al.* (2020) who observed down-regulation of PPR containing genes in leaves of grape variety 'Jingxiangyu' with increase in temperature.

Grape is an important fruit crop worldwide with a high commercial value. In recent years, extreme weather events during critical phases of growth and development results in unfavorable environmental conditions for grape resulting in reduced yield and quality and long term effects on vine productivity and life. Recurrent heat-wave like conditions with temperatures above 40 °C or more, are becoming frequent in many grape growing regions resulting in heavy economic losses. Heat stress is often coupled with other stresses like oxidative stress, UV radiation etc. Under such extreme conditions, proper management of vineyards to mitigate the adverse effect of heat stress through irrigation and/or canopy management is crucial for sustained yield. Understanding of plant response to management practices will enable long-term strategies for sustained grape yield and productivity under changing climatic conditions. We analyzed response of grapevine to field heat wave condition under varied irrigation regimes. We observed that heat stress has the largest effect on the vine and expression of a large number of genes was modulated due to heat stress as compared to moisture. Deficit irrigation (80 % of recommended) did not elicit significant molecular response in 'Fantasy Seedless' indicating that vines are not experiencing the stress even at reduced level of irrigation. The differential response of 'Fantasy Seedless' to deficit irrigation at biochemical levels have been reported by Shetty *et al.* (2019) and yield was not affected by reducing the irrigation by 20 %. Our results further support that this variety has better water use efficiency. Similarly we observe that sub-surface irrigation helped the vine to overcome the stress through modulation of some genes. The sub-surface irrigation works on the principle of delivering irrigation water directly in the root zone, thereby, reducing the energy consumed by the roots in the process of search for water. This irrigation technique results in saving of up to 25 % irrigation water under semi-arid regions of grape growing (Sharma and Upadhyay 2011). The genes identified in response to moisture as well as heat stress are good candidate genes for introducing multiple stress tolerance.

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