Final Scientific Report

Cover Page

BARD Project Number: BARD # IS-4325-10

Date of Submission of the report: May 2014

Project Title:

Toward improved grape nutrition and defense: the regulation of stilbene metabolism under drought

Investigators

Institutions

Principal Investigator (PI): Aaron Fait, Ben Gurion University of the Negev, Israel

Co-Principal Investigator (Co-PI): Grant Cramer, University of Nevada (UNR), Reno, Nevada, USA

Collaborating Investigators: Avichai Perl, ARO, Israel

Metabolomics, proteomics, polyphenols, RNAseq, resveratrol, grape berry development

Keywords *not* appearing in the title and in order of importance. Avoid abbreviations.

Abbreviations **commonly** used in the report, in alphabetical order:

Budget: IS: \$ 132,000 US: \$ 168,000 Total: \$ 300,000

Signature
Principal Investigator

Signature
Authorizing Official, Principal Institution

Final Scientific Report

Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted) BARD support acknowledged			1	1
Submitted, in review, in preparation	3			3
Invited review papers	1			1
Book chapters				
Books				
Master theses			1	1
Ph.D. theses		1	2	3
Abstracts	3		3	6
Not refereed (proceedings, reports, etc.)				

Postdoctoral Training: List the names and social security/identity numbers of all postdocs who received more than 50% of their funding by the grant.

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings	2	2		3
Longer Visits (Sabbaticals)				

Description Cooperation:

The PIs collaborated by sharing samples harvested in the two countries and performing complementary analysis on the plant material. The US invested in transcript analyses and protein profiling, the Israeli team implemented metabolite based analysis of central and specialized metabolism, as well as investigating the physiology of the plant response. The experimental designs in the two countries were overall similar but had different focuses. In the US, the varietal response was favored; in Israel the developmental aspect of the berry, the response to stress of the leaf and of the berry received much attention. In Israel also the berry derived cell culture experiments were established with the collaboration of Avichai Perl.

Patent Summary (numbers)

	Israeli inventor only	US inventor only	Joint IS/US inventors	Total
Submitted				
Issued (allowed)				
Licensed

Final Scientific Report

Abstract

The goals of the present research proposal were to elucidate the physiological and molecular basis of the regulation of stilbene metabolism in grape, against the background of (i) grape metabolic network behavior in response to drought and of (ii) varietal diversity. The specific objectives included the study of the physiology of the response of different grape cultivars to continuous WD; the characterization of the differences and commonalities of gene network topology associated with WD in berry skin across varieties; the study of the metabolic response of developing berries to continuous WD with specific attention to the stilbene compounds; the integration analysis of the omics data generated; the study of isolated drought-associated stress factors on the regulation of stilbene biosynthesis *in planta* and *in vitro*.

Background to the topic

Grape quality has a complex relationship with water input. Regulated water deficit (WD) is known to improve wine grapes by reducing the vine growth (without affecting fruit yield) and boosting sugar content (Keller et al. 2008). On the other hand, irregular rainfall during the summer can lead to drought-associated damage of fruit developmental process and alter fruit metabolism (Downey et al., 2006; Tarara et al., 2008; Chalmers et al., 1992). In areas undergoing desertification, WD is associated with high temperatures. This WD/high temperature synergism can limit the areas of grape cultivation and can damage yields and fruit quality.

Grapes and wine are the major source of stilbenes in human nutrition, and multiple stilbene-derived compounds, including isomers, polymers and glycosylated forms, have also been characterized in grapes (Jeandet et al., 2002; Halls and Yu, 2008). Heterologous expression of stilbene synthase (STS) in a variety of plants has led to an enhanced resistance to pathogens, but in others the association has not been proven (Kobayashi et al., 2000; Soleas et al., 1995). Tomato transgenic plants harboring a grape STS had increased levels of resveratrol, ascorbate, and glutathione at the expense of the anthocyanin pathways (Giovinazzo et al. 2005), further emphasizing the intermingled relation among secondary metabolic pathways. Stilbenes are induced in green and fleshy parts of the berries by biotic and abiotic elicitors (Chong et al., 2009). As is the case for other classes of secondary metabolites, the biosynthesis of stilbenes is not very well understood, but it is known to be under tight spatial and temporal control, which limits the availability of these compounds from plant sources. Only very few studies have attempted to analyze the effects of

Final Scientific Report

different environmental components on stilbene accumulation (Jeandet et al., 1995; Martinez-Ortega et al., 2000). Targeted analyses have generally shown higher levels of resveratrol in the grape skin (induced), in seeded varieties, in varieties of wine grapes, and in dark-skinned varieties (Gatto et al., 2008; summarized by Bavaresco et al., 2009). Yet, the effect of the grape variety and the rootstock on stilbene metabolism has not yet been thoroughly investigated (Bavaresco et al., 2009).

The study identified a link between vine hydraulic behavior and physiology of stress with the leaf metabolism, which the PIs believe can eventually lead to the modifications identified in the developing berries that interested the polyphenol metabolism and its regulation during development and under stress. Implications are discussed below.

Achievements

Grapevine berries undergo complex biochemical changes during fruit maturation, many of which are dependent upon the variety and its environment. In order to elucidate the varietal dependent developmental regulation of primary and specialized metabolism, berry skins of Cabernet Sauvignon and Shiraz were subjected to gas chromatography–mass spectrometry (GC-MS) and liquid chromatography–mass spectrometry (LC-MS) based metabolite profiling from pre-veraison to harvest. The generated dataset was augmented with RNAseq-transcript profiling. The analysis of the metabolite data revealed similar developmental patterns of change in primary metabolites between the two cultivars. Nevertheless, towards maturity the extent of change in the major organic acid and sugars and precursors of aromatic and phenolic compounds was varietal specific including a varietal-dependent regulation of the specialized metabolism. Enhanced stress related metabolism, e.g. trehalose, stilbene and ABA in Shiraz berry-skin corroborate its relatively higher susceptibility to environmental cues. Correlation based analysis confirmed the tight coordinated metabolic changes during development, and suggested a source-sink relation between the central and specialized metabolism, stronger in Shiraz.

Drought and its implications on plant physiology in two contrasting varieties

First, drought stress is known to limit photosynthesis rates and to inflict photo-oxidative damage in grapevines. Grapevines, which are considered drought-tolerant plants, are characterized by diverse hydraulic and photosynthetic behaviors, depending on the cultivar. This first part of the research compared the photosynthesis and the photorespiration of Cabernet Sauvignon (Cs) (isohydric) and Shiraz (anisohydric) in an attempt to acquire a wider perspective on the

Final Scientific Report

iso/anisohydric phenomenon and its implications. Cs was found to behave in a near isohydric manner whereas Shiraz behaved in a near anisohydric manner. Compared to Shiraz, the reduced stomata conductance values of Cs were accompanied by higher water use efficiency and photorespiration rates, as well as photosystem II photochemical potential (F_v / F_m). As compared with Shiraz, Cs compensated for lower stomata conductance by higher photosynthesis and photorespiration. These two processes contributed to higher electron flow rates that might have a role in photoinhibition avoidance, which was observed in the stability of F_v / F_m under drought stress.

Grapevine response to drought, the plant and its metabolism

Results showed that *Vitis vinifera* cultivars possess a common metabolic response to water deficit. Central metabolism, and specifically N metabolism, plays a significant role in stress response in vine. At the cultivar level, Cabernet Sauvignon was characterized by milder metabolic perturbations, likely due to a tighter regulation of stomata upon stress induction. Network analysis highlighted the cultivar specificities in respect to metabolic alteration induced by stress and identified metabolites with a significant structural and likely biological role in vine stress response.

Grapevine response to drought, the berry and its metabolism

In the present part of the project, the cultivar variability in metabolic regulation of grape berries under deficit irrigation was investigated. Shiraz and Cabernet Sauvignon (CS) were grown under two irrigation treatments and their berry skin metabolism was analysed by parallel GC/MS and LC/MS at four development stages. Metabolic modifications, notable at the early stages of the season, were buffered toward maturity in both cultivars. Phenol metabolism in response to water stress underwent significant modifications, unique to each cultivar. For example, at harvest, in CS a decrease in flavanols and increase of flavonols were noticeable, while in Shiraz anthocyanin accumulation was measured.

Varietal analysis of proteomics and metabolomics changes in berry skins in response to progressive water deficit

Comparative label-free shotgun proteomic analysis was used to assess grape berry skin protein abundance differences amongst five grapevine cultivars, three red skinned and two white skinned varieties (Cabernet Sauvignon, Merlot, Pinot Noir, Chardonnay and Semillon), exposed to a progressive water deficit treatment during the 2011 growing and harvest season. The physiological data (stem water potential,

Final Scientific Report

berry diameter, titratable acidity and sugar levels) indicated that vines were exposed to moderate water stress during the growing season. Shotgun proteomic analysis of grape berry skin tissue at maturity identified 2,874 proteins across five grapevine cultivars, with an average CV of 7% for the protein abundance of all proteins. A reliable common core set of 1,211 proteins with all varieties was quantified in each replicate, variety and treatment. The protein abundance of proteins affecting the quality of the fruit at harvest (flavonoid and terpenoid biosynthesis) were affected by water deficit. Other observed changes influenced the available substrate for yeast fermentation by the physical crushing of the berry in wine production (fatty acid and amino acid metabolism). Pathogenesis-related proteins represented an additional class of highly abundant skin proteins, representing ~4% of all proteins identified. The relative metabolic content from berry skins of primary and specialized secondary metabolites were also analyzed by GC-MS and LC-MS based methods. Some amino acids and phenylpropanoids were significantly affected by water deficit treatment in each of the varieties. Deficit irrigation induced metabolic differences that were unique for each variety.

The above data is currently being summarized and integrated with parallel metabolite profiling that was undertaken on the collected samples. It is also being assembled in a manuscript. The abstract is given at the end of this report.

Implications, both scientific and agricultural.

From the study it can be concluded that grapevine varieties as close as Cabernet sauvignon and Shiraz vary in their physiology of stress. This diversity can lead to varietal specific alterations in the berry metabolism. Cultivars with a stronger isohydric behavior can maintain leaf metabolism balanced in spite of environmental alterations. The relation between physiology and leaf metabolism can be eventually expressed in the fruit metabolism, specifically in those metabolites related to stress, including the stilbenes. Specialized metabolism was also shown to be significantly affected by environment X cultivar interaction. The implications of the results are that specific varieties should be chosen for best performances in more or less stable environments and for defined purposes (wine making Vs stilbene production). The present study also identified few branching enzymes in the polyphenol metabolism modulating the anthocyanin profile that are highly specific to the cultivar and could be used for enhancing grape quality. Last the use of berry derived cell culture showed that individual stresses related to drought do have a differential effect on the

Final Scientific Report

modulation of the branched polyphenol metabolism. Results of this last part of the research are currently being summarized.

Details of cooperation:

The PIs collaborated by sharing samples harvested in the two countries and performing complementary analysis on the plant material. The US invested in transcript analyses and protein profiling, the Israeli team implemented metabolite based analysis of central and specialized metabolism, as well as investigating the physiology of the plant response. The experimental designs in the two countries were overall similar but had different focuses. In the US, the varietal response was favored; in Israel the developmental aspect of the berry, the response to stress of the leaf and of the berry received much attention. The integration of the results was made possible by the mutual visits of students between the two laboratories. During their stay students learned the respective technologies and complemented with their knowhow their counterparts. This led to an enhanced integration of the metabolite and RNAseq data and eventually as well as the optimization of network analysis tools for the integration of proteomic data and metabolites. In collaboration with Avichai Perl, the Fait team in Israel established the berry derived cell culture experiments. The cultures were exposed to different individual environmental stresses, profiled for their metabolic phenotype and analyzed for the levels of target transcripts within the polyphenol metabolism. The results are currently being summarized and will be submitted for publication within the end of 2014.

• List of Publications

- Hochberg U, Degu A, Fait A, Rachmilevitch S (2012) Near isohydric grapevine cultivar displays higher photosynthetic efficiency and photorespiration rates under drought stress as compared with near anisohydric grapevine cultivar, *physiologia plantarum*, 147,443-352
- Hochberg U, Degu A, Toubiana D, Gendler T, Nikoloski Z, Rachmilevitch S, Fait A (2013) Metabolite profiling and network analysis reveals coordinated changes in grapevine water stress response. *BMC plant biology*, 13,184
- Asfaw Degu, Uri Hochberg, Noga Sikron, Luca Venturini, Genny Buson, Ryan Ghan, Inbar Plaschkes, Vered Chalifa-Caspi, Fulvio Mattivi, Massimo Delledonne, Mario Pezzotti, Shimon Rachmilevitch, Grant R Cramer, Aaron Fait (BMC plant biology, being revised). Metabolite and transcript profiling of berry skin during fruit development elucidates differential regulation between Cabernet Sauvignon and Shiraz cultivars at branching points in the polyphenol pathway.

• Appendices (start with the next page)