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## **Forward**

Grape is one of the most widely cultivated fruit crops, covering about 20 million hectares of arable land, and demand has been steadily increasing over the last 15 years, even in countries not traditionally associated with large acreages of wine grapes. That being said, a large portion of the world's wine-producing areas are located in regions either currently suffering from water deficits or in ones expected to suffer them in the future, and where seasonal drought coincides with the grapevine-growing season, limiting grapevine yield and wine quality. "Climate change" is a fact and presents a challenge to the grape and wine industry in how to cope with increasing environmental unpredictability. Hence, more than ever before, there is a need to understand the physiological mechanisms and the genetic background underlying the interactions between the vine and its environment, and to focus on innovating alternative vineyard management practices for the development of sustainable, quality products under changing climatic conditions.

A landscape of grapevines growing in the middle of the desert is almost surrealistic. However, grapes (*Vitis vinifera* L.) have been cultivated and fermented into wine for more than 7,000 years in the Middle East. Shivta is a picturesque ancient (300 BC) Nabatean city in the northern Israeli Negev Desert and one of the most well-preserved archeological sites in Israel considering its size. Shivta, like other nearby Nabatean cities, hosts two wine presses, one of them located just opposed to the plaza. This is one of many examples of archeological evidence indicating high levels of wine production in the ancient Negev Plateau\*. While the average potential evaporation for this area is 2042.2 mm/yr, the average yearly rainfall is 87.6 mm, with no rain occurring in the grapevine-growing season. In contrast, due to the elevation of the Negev Plateau (400-600 masl), there is a measured average of 200 days a year with the presence of dew that contributes a total amount of about 40 mm of water, which accounts for over 30% of the annual precipitation in this region. The region is marked by temperature extremes, with a 15 to 20°C difference between day and night. In spite of this harsh environment, a rich antique wine industry likely exported wines from the Negev Plateau area to Greece, Egypt and Turkey. In recent years, the Negev Plateau has reclaimed its place as one of the leading areas of the Israeli wine industry.

The expected increase in arid lands worldwide and the unpredictability currently characterizing the Mediterranean climate encourage us to look at the Negev Plateau as a model area for grape cultivation in future scenarios.

This first Italy-Israel grapevine research symposium, GRACious, aims to motivate new efforts to meet the challenges of modern viticulture in the Mediterranean basin and to foster collaboration between Israeli and Italian scientists in the field. This symposium's occurrence was initially inspired by Luigiterzo Bosca, who visited Sede Boqer six years ago. During his Israeli visit, Bosca was unequivocal about the need to foster desert viticulture and its research. In 2010, the Bosca Company, the Genomic Research Centre of Fiorenzuola d'Arda, Phenom Networks Ltd., BG Negev Technologies & Applications Ltd. and the Jacob Blaustein Institutes for Desert Research (BGU) initiated a Eureka-labelled project funded by the Israeli Ministry of Industry Trade and Labor and by the Italian Foreign Ministry to rediscover the chemical complexity of the Bosca Muscat collection. Luigiterzo Bosca was General manager of Bosca spa of Canelli since 1973, of which he was the President from 1988 till his retirement in 2005, until recently he retained the position of Honorary Chairman of the Board. He passed away this year. To his person and vision this conference is dedicated.

Aaron Fait, Israel 2014

\* suggested reading: Hadas, A. 2007. The Vine & Wine – Archaeology in The Land of Israel. Korenberg Publishing Co., Israel

## **Breeding project in Israel for the improvement of table grapes**

*Dgani O., Dinkin I., Okon T., Sarafian A. and Perl A.*

Approximately 80% of the table grapes sold worldwide is seedless. We anticipate that in the years to come, most markets will prefer seedless cultivars. The international market is looking for complete seedlessness, as in the Thompson cultivar. The size of the seed rudiment is playing a major role in the markets. Grape with small to medium seed rudiment, even if they are soft and edible, risk to be classify as “soft seeded” thus rendering the producer less income compared to truly seedless grapes. The world is currently flooded with many new seedless grapes. However, only premium excellent cultivars with very good eating qualities will survive and will be a success on the markets. All the others will disappear or become niche cultivars. This assumption is heading our breeding strategies and parent selection for crosses. We dedicated years in trying to develop grape cultivars that will replace traditional cultivars, having significant improvements over existing cultivars. We tried and continue to try to develop the "Game Changer". The specific objectives in our breeding programs for table grape can be summarized as follows: Seedlessness; Novel tastes and aromas; Better eating quality; Attractive appearance of large berries, bright colors, firm flesh all in naturally loose and uniform bunches. It is also expected that the health giving benefits of the polyphenols and antioxidants in the colored grapes will become a special market for interesting new health promoting selections. Improved technologies and strategies will be exemplified in the lecture such as: Improvements in the embryo rescue procedures, improvements in the selection of potential parents for crosses and novel protocols for growing new cultivar in order to obtain the best performances in the field. Several new and promising selections recently developed are presented.

## **From one to the many genomes of grapevine: The evolution of the grapevine pan-genome**

*Morgante M.*

The analysis of variation in plants has revealed that their genomes are characterised by high levels of structural variation, consisting of both smaller insertion/deletions, mostly due to recent insertions of transposable elements, and of larger insertion/deletion similar to those termed in humans Copy Number Variants (CNVs). These observations indicate that a single genome sequence might not reflect the entire genomic complement of a species, and prompted us to introduce the concept of the plant pan-genome, including core genomic features common to all individuals and a Dispensable Genome (DG) composed of partially shared and/or non shared DNA sequence elements. The very active transposable element systems present in many plant genomes may account for a large fraction of the DG. The mechanisms by which the CNV-like variants are generated and the direction of the mutational events are still unknown. Uncovering the intriguing nature of the DG, i.e. its composition, origin and function, represents a step forward towards an understanding of the processes generating genetic diversity and phenotypic variation. Additionally, since the DG clearly appears to be for the most part the youngest and most dynamic component of the pan genome, it is of great interest to understand whether it is a major contributor to the creation of new genetic variation in plant evolution as well as in the artificial selection processes of plant breeding. We have resequenced to high coverage more than 50 grapevine accessions and used a variety of approaches to detect SNPs as well as structural variants of different size and origin, including de novo assembly of a selected set of genotypes. We will discuss the extent and composition of the pan genome in grapevine, the different mechanisms that generate and maintain the dispensable portion, the epigenetic and phenotypic effects of the DG and the rates and modes of creation of new genetic variation due to DG components.

## **Effective manipulation to increase health promoting metabolites in a red grape cell suspension culture**

Oren-Shamir M., Manela N., Oliva M., Perl A., Galili G., Ovadia R.

Grapes (*Vitis vinifera*) are important sources of phyto-antioxidants, pigments, and essential nutrients worldwide. There is incredible diversity in chemical composition within the various genotypes of grapes, a significant part of which is derived from the aromatic amino acids (AAAs). Various studies showed that consumption of grape products can reduce the incidence of chronic illnesses, such as cancer, cardiovascular diseases, ischemic stroke, neurodegenerative disorders and aging. One of the main bioactive phytochemicals synthesized by grapes is phenylpropanoids. Within the phenylpropanoids, considerable attention has focused on the antioxidant activity of a variety of phenolic compounds, especially stilbenoids anthocyanins and flavonols, which are considered the major components of phyto-antioxidants responsible for the “French-Paradox” characteristics of the grapes. In order to enhance phenylpropanoid production we transformed a red grape cell suspension culture with a feedback insensitive form of the bacterial *AroG\** gene, encoding for DAHPS enzyme, the first enzyme in the shikimate pathway. Metabolic analysis of the transformed lines shows a dramatic increase in the concentrations of Phe in these cells. In addition, the three main metabolic pathways originating from Phe in grape berries, benzenoid, flavonoid and stilbene pathways seem to be 'open', at least partially, since we detect a significant increase p-salicylic acid, dehydroquercetin and resveratrol respectively.



## **Role of genetic, environmental and cultural factors on stilbene synthesis in the grapevine**

*Bavaresco L., Morreale G., Flamini R., Maoz I., De Rosso M.*

Stilbenes are a class of phenolics deserving particular attention due to their role in the plant as phytoalexins and in humans as compounds claimed to have beneficial effects on health. Stilbene phytoalexins accumulate in soft tissues of the vine and they are produced by the phenylpropanoid pathway. Stilbene synthase (STS) is the key enzyme and it produces resveratrol, the basic monomer which can be glycosylated, hydroxylated, methylated or converted in more complex compounds (dimers, trimers, tetramers). According to the grapevine genome sequencing, STS is encoded by a gene family of many highly similar paralogues and recent data account for their expression in different grapevine organs. At leaf and berry (skin) level, stilbenes are produced after the occurrence of a biotic and/or abiotic stress. Disease resistant genotypes produce very quickly high concentrations of stilbenes, while disease susceptible ones slowly produce low concentration of those compounds. Biotic and abiotic factors are listed and some examples of the triggering ability of some of those are described. Under the same elicitation pressure, the final concentration of stilbenes in grapes and wine is affected by viticultural and oenological factors, such as the grape variety, the meteorological conditions, the soil, the cultural practices, the wine-making procedures. Study of metabolomics allows identifying a large array of stilbenes in grapes and wine, that could have major implication for health. The health claims for resveratrol are discussed, spanning from the antioxidant to the anti-cancer effects, with an emphasis on the lifespan extension, by triggering the Sir/SIRT genes.

## **Rootstock-scion interaction in grapevine: An omics perspective**

*Mica E., Faccioli P., Tononi P., Delledonne M., Storch P., Zombardo A., Morreale G., Vaňková R., Dobrev P.I., Mocali S., Bernardo L., Giusti L., Costantini E., Priori S., Crosatti C., Reshef N., Or E., Fait A., Cattivelli L.*

Rootstock selection improves the vineyards in terms of grape quality and quantity, influencing water uptake, stress resistance and plant vigor. Nevertheless, poor genomics evidences have been reported so far linking rootstock-scion interaction with the molecular mechanisms involved in berry maturation.

Pinot Noir vines were grafted in 2005 on two distinct rootstocks (Paulsen 1103 and M 101-14) and grown in pots where soil water tension and temperature were monitored continuously. Non grafted PN plants, grown in the same conditions, have been used as control. Berries have been collected in three biological replicates, at veraison and harvest. Different analyses have been performed on berries: mRNA and small-RNA next generation sequencing, LC-MS based metabolomic analyses and physiological assays. Hormonal analyses have been performed to evaluate cytokinin and auxin levels. Furthermore the structure of rhizospheric bacterial community has been assessed by means of Denaturing Gradient Gel Electrophoresis (DGGE). A complete transcriptomic scenario has been described for developing berries. Main transcriptional changes have been observed when comparing grafted vs. non grafted plants, while minor fluctuations have been observed between rootstocks. A similar trend has been observed when analyzing their metabolomic properties with main differences in the polyphenolic contents and acidity of mature berries. The less vigorous rootstock increases the cytokinin level in the buds. The rhizosphere analysis showed a higher bacterial community structure associated to grafted plants. The highest bacterial diversity was associated to the most vigorous rootstock. Grafting strongly influences the molecular mechanisms of berry development, although the overall effect of different rootstock examined in non-stressed conditions is not incisive.

## **Physiological mechanisms involved in production and quality of fruit and wine**

*Bravdo B.*

Production of fruit is the final outcome of the rate and interaction between many physiological processes that are controlled by genes and by environment. Advanced agriculture provides means for affecting the activity of the numerous physiological processes aiming at optimizing the production and quality of the yields produced. One of the major breakthroughs of the century for many agricultural crops but particularly for the worldwide viticulture is the invention and application of drip irrigation. Drip irrigation greatly contributed to our understanding of roots physiological functions such as water and mineral absorption, growth and growth regulators production as well as water relations and vegetative and reproductive growth. Due to the slow mobility of Phosphorous in the soil, application of soluble P via drip was found to enable P petiole content at optimal levels and thereby increase production and quality. Controlled drip irrigation enabled to regulate the vegetative growth throughout the major stages and thereby achieve a proper reproductive to vegetative growth, namely crop load. The use of pruning weight and leaf area measurements enabled to determine and define the terms 'under cropping', 'optimal cropping' and 'over cropping'. These values may vary between varieties x rootstocks combinations as well as trellising systems. The use of comprehensive drip fertigation may help adjusting some field variations. Production and quality of wine grapes showed that The 140 Ruggeri rootstock was found to perform better than salt creek as well as some other rootstock under saline conditions. The effect of salinity and regions on aroma compounds synthesis will be presented.

## **Influence of Seed on the ripening process of Grape Berry**

*Gouthu S., Di Y., Megraw M., Morré J., Maier C., Vondras A., and  
Deluc L.*

We used the asynchronous cluster as research model to design three separate studies aimed to better understand the influence of seed on the ripening process. Using genome-wide technology, the first study was aimed to determine whether the ripening asynchrony persists until maturity. The second study was conducted to ascertain the influence of flowering time in determining the level of ripening asynchrony in a grape cluster at the ripening onset (véraison). The third study was meant to evaluate the seed contribution to the ripening states of berries at véraison. Our findings suggest 1) the existence of a natural mechanism that reduces intra-cluster berry variability toward maturity via an enhancement of the ripening rate in lagging berries, 2) flowering time is not the only prevailing factor to determine when a berry will enter into the ripening phase. Seed mass relative to berry was found to be a better predictor of the ripening status of individual berries, and 3) measurement of expression of auxin and ABA-related genes along with hormone quantification, revealed that véraison berries with high seed weight to berry weight ratio (S:B) perturbs the auxin homeostasis in the pericarp resulting in the delay of their entry into the ripening. Our phenological observation also suggests that berries with high S:B ratio are also the berries able to catch up toward maturity, which likely suggests that seeds might also be associated with the later stages of the ripening process.

## **To die for: the price of long-term exposure of grapevines to salinity**

*Ben-Gal A.*

Low quality water is frequently utilized for irrigation in water scarce regions, and consequently, grapevines are exposed to salinity. Long term response of grapevines was examined in two desert research and development stations. Table grapes (Sugraone) in the Southern Arava R&D Center in Yotvata were exposed to six salinity levels for a single season in lysimeters and to four salinity levels for five years in a vineyard. Wine grapes (Cabernet Sauvignon) grown on four different root stocks and irrigated with three salinity levels were studied for 3 years in The Negev Highlands R&D Center.

Response of table grapes to salinity was observed to involve two mechanisms: (i) a reduction in transpiration and growth which began as soon as salinity was experienced; and (ii) vine mortality which was correlated with salinity level, a sharp increase in Na and Cl content of leaves, and time .

Rootstocks of wine grapes differentially excluded Na and Cl from vines with some allowing more accumulation of Na and others more of Cl in plant tissues. While rootstock was not found to influence growth or yield, the ability to exclude Na and Cl from shoots and fruit was found to a) increase wine quality by reducing ion salt levels in must and b) reduce or postpone mortality rates.

Irrigation of grapevines with water high in salts reduces productivity and vineyard life expectancy and reduces wine quality. Additional, environmental, considerations of water needed to leach salts and of leachate disposal, make desalination an attractive option for vineyard irrigation.

## **SERRES: An Italian project for grapevines rootstock breeding and selection**

*Failla O., Brancadoro L., Espen L., Scienza A.*

Rootstock in viticulture plays a central role in the plant adaptation to limiting soil and climatic conditions. Ideally, in these environments, the suitable rootstock should guarantee an adequate growth and physiological functionality to assure high yields and quality grapes.

In 2013, four new rootstocks have been released and registered in the Italian Catalogue of Varieties admitted to cultivation with the name of M1, M2, M3 and M4. Out of which three have improved performance in relation to water stress (M4), lime-induced iron chlorosis (M1, M2), salt stress (M4, M2) resistances. The other one (M3) has very low vigor and low nitrogen uptake ability. A large field testing, in different environmental conditions, proved the ability of the four new obtainments, in comparisons to six commercial reference rootstocks, to induce high and stable yields in conjunction with proper grape ripening with high enological profiles.

During the last three years, a large collaborative project titled “Selection of new grape rootstocks resistant to abiotic stresses through the development and validation of physiological and molecular markers – SERRES” allowed, among a wider range of research activities, to characterize the physiological response of M4 rootstock to the water deficit. M4 exhibited a greater capacity to tolerate both WS and exposure to an increasing concentration of Na and Cl, maintaining photosynthetic activity also under severe stress conditions.

Moreover, a phenotyping trial to characterize the vine response to water deficit for association genetic studies, has been developed and tested on a core collection built on *Vitis* genus rootstocks accessions.

## **The collection and initial characterization of Israel's native grapevine population**

*Drori E., Henig Y., Rahimi O., Lorenzi S., Marrano A., Brauner H., Failla O., Grando M.S.*

The holy land has a long history of winemaking, widely mentioned in ancient scripts. The Muslim occupation of this region in the 7th century started a long period of wine consumption prohibition, resulting in the eventual loss of local wine varieties. Thus, the renewing Israeli wine industry is based solely on international varieties. In the last three years we are collecting and assessing for the first time a wide Israeli grapevine germplasm collection. Our efforts are in two directions: Collection of local cultivated varieties, and adding new ones by a wide survey done all over Israel. We collect both *Sylvestris* and *Sativa* populations. Most importantly, we strive to identify varieties suited for quality wine production. A collection vineyard is being planted with 6 plants per unique accession. By now we collected 270 accessions. 148 accessions were analyzed by 10 SSR loci, and we found 61 unique profiles which were analyzed against European databases. Out of these, 10 were identical to Israeli and Palestinian accessions. To understand the relation in this group and to other collections, we conducted an initial population analysis. The analysis revealed two main groups, one of 35 accessions, mostly *V.v. ssp. Sativa*, and a second of 15 accessions, all *V.v. ssp. Sylvestris*. In addition, Primary and secondary descriptors, Phenophase description, and some eno-carpological traits were obtained for most of the accession, resulting in the identification of at least 3 white and 3 red *Sativa* varieties suited for quality wine production. The wilds in Israel contain wide populations of both *V.v. ssp. Sativa* and *Sylvestris*. We believe that by collecting and assessing these populations, an array of new-old varieties would be presented, starting a new era in the Israeli wine industry.

## **Exploring a large grape germplasm collection for genetic association studies**

*Grando M.S., Costantini L., Lorenzi S., Moreno-Sanz P., Marrano A., Nwafor C.C., Prazzoli M.L., Yu X., Battilana J., Grzeskowiak L.*

Quantitative trait loci (QTL) linkage mapping is an effective tool for the identification of genetic loci underlying natural variation, and several attempts to dissect the architecture of target traits for breeding have been reported in grapevine. Recently, genome-wide association studies (GWAS) have received increased attention for the identification of QTLs in plants as an alternative to, or in combination with linkage mapping. Candidate gene association studies are a complementary extension of GWAS and focus the association analysis exclusively on a selection of genes with known or potential functions in the trait of interest. The candidate gene approach has the potential to narrow down the set of trait associations, and was confirmed to be successful in our experiments to identify the gene and even the individual nucleotides that affect the expression of Muscat flavor in cultivated grapevines.

The objectives of our latest studies were to detect the quantitative trait genes underlying natural variation of key developmental stages (budburst, flowering and the onset of ripening) as well as of anthocyanin content and composition of the berries in *Vitis vinifera*. We used traditional linkage mapping followed by a candidate gene association analysis in panels of multiple grapevine accessions, sorted from a large germplasm collection (2.300 accessions) whose genetic diversity and structure were previously characterized by SSR and SNP markers. Specific genes with proven or suspected activity, for example in flowering time control, are also explored in a broader range of plant materials, to look for variants that may have been selected during domestication.



## **Towards control of bud dormancy release in arid zones**

*Zheng C., Halaly T. and Or E.*

In warm winter regions, where the table grape industry preferentially is located, artificial induction of bud dormancy release is mandatory for coordinate, early production of economical grape yields. The single effective artificial stimulus available for commercial use in vineyards is hydrogen cyanamide (HC). Unfortunately, its ability to induce respiratory stress, which initiates a biochemical cascade that leads to effective dormancy release, is also responsible for its toxicity, both to the vines and within the environment. The development of safe alternatives for artificial induction of bud dormancy release is essential due to the initiative to ban its use in the near future. Such task requires comprehensive understanding of the cascade of biochemical changes that is induced by the currently available artificial stimuli of grape bud dormancy release. Our genomic studies recently led to the development of a working model for such cascade. Our post genomic studies, which have affirmed the predictive power of the model, support a central role for ethylene and ABA in regulation of dormancy release and question the involvement of Gibberellins, These studies will be discussed.

## **Water shortage and grape quality: is it a fruitful relationship?**

*Peterlunger E*

Grapevine is a species naturally adapted to poor soils and not much water availability, due to the environment where it evolved (Caucasian mountains, dry climate). The cultivation has spread all over the world, even in very fertile areas where the growth is too abundant for the natural habit of the plant, leading to luxurious vegetation, heavy production but poor quality. So the best production in terms of quality (and therefore of price of the bottle) is obtained via a moderate water availability. The quantification of this is crucial, i.e. to find the level which is giving rise to better quality, namely better secondary metabolites, and among them in particular better polyphenolic compounds. Several studies have been conducted in various countries, using different parameters. A good level of stress has been determined through measurement of plant water status with leaf water potential; the level is more negative in red varieties, less negative in white ones. Furthermore this level may vary in relation to variety and area. Water shortage is triggering gene expression, especially in red varieties for color synthesis (anthocyanin production). Also the proportion of different anthocyanins has been shown to be modified by water shortage, thus inducing a different color in grapes and wine: a higher proportion of tri-substituted anthocyanins is produced under water shortage, giving a nuance to berry and wine more blue-purple, less red-orange, which is appreciated by the consumer. Tannins as well (especially proanthocyanidins) can be improved by moderate water shortage. So, yes: this relationship can be fruitful.

## **LAI –K<sub>c</sub> relations as the base for skilled regulated deficient irrigation for quality red wine**

*Netzer Y., Munitz S. Dotan D., Hayat Y., Drori E. and Schwartz A.*

Water consumption (ET<sub>c</sub>) of grapevines (*Vitis vinifera* cv. Cabernet Sauvignon) trained to a vertical shoot positioning trellis system was measured during three growing seasons (2012-2014) using 6 automatic drainage lysimeters. The lysimeters (1.5 m<sup>3</sup> each) were installed in commercial vineyard in a semi-arid region in the center mountain region, Israel. Reference evapotranspiration (ET<sub>o</sub>) was calculated from regional meteorological data according to the Penman Monteith equation. Seasonal curves for the crop coefficient (K<sub>c</sub>) were calculated as  $K_c = ET_c / ET_o$ . Maximum ET<sub>c</sub> values in different seasons were 7.5 and 6.64 mm day<sup>-1</sup> and seasonal ET<sub>c</sub> (from DOY 99 through DOY 288) were 702 and 790 mm for 2012-2013 respectively. Leaf area index (LAI) was measured weekly using the SunScan Canopy Analysis System. Maximum LAI were 1.36 and 1.16 m<sup>2</sup> m<sup>-2</sup> for the 2012-2013 seasons respectively. The seasonal pattern of LAI was similar to the control vines grown in the vineyard. A linear curve relating K<sub>c</sub> to LAI (R<sup>2</sup> values were 0.6 and 0.82) is proposed as the basis for efficient irrigation management. The difference in ET<sub>c</sub> and K<sub>c</sub> values that were observed differed from those obtained in table grapes (Netzer et al. 2009; Williams and Phene 2003) and are explained by the different LAI and canopy architecture.

Application of the LAI-K<sub>c</sub> method was used in the past 4 seasons (2011-2014) in Yatir region for irrigation of Cabernet Sauvignon and Shiraz grapevines. A significant yield increase was recorded in both cultivars (compared to local irrigation regime) with no significant effect on wine quality.

## **Genotype by environment in *Vitis vinifera*: The genomic approach**

*Zenoni S., Dal Santo S., Fasoli M., Massonet M., Brancadoro L., Scienza A., Tornielli B.G., Pezzotti M.*

The phenotype (P) is the result of the interaction between the genetic composition of the plant (G) and the environment (E). To produce suitable genotypes for multiple environments the interaction GXE should be accounted and assessed for each plant species. Plants are sessile, they cannot migrate when challenged by fluctuation in environmental conditions therefore adaptation strategies should be activated to cope with environmental heterogeneity. A fundamental strategy is to change the phenotypic expression, this phenomenon is called “phenotypic plasticity”.

Phenotypic plasticity refers to the range of phenotypes a single genotype can express as a function of its environment. When phenotypic plasticity differs between genotypes, this is described as GXE. Although phenotypic plasticity is an important ecological phenomenon, the underlying genetic and molecular mechanisms remain still poorly characterized. The availability of high-throughput expression profiling technologies now makes it possible to analyze gene expression on a global scale, so that transcriptome plasticity can be investigated directly. We investigated the extent to which phenotypic plasticity in grape berries reflects underlying changes in the transcriptome by using microarray technology to study global gene expression profiles. of: 1) a single clone of *Vitis vinifera* cv Corvina cultivated in different vineyards and harvested at different developmental stages over 3 consecutive years; 2) two grapevine varieties, in three environments during four developmental stages of berry development over 2 consecutive years. We studied the relationships among differential gene expression profiles, growing conditions and ripening parameters and identified several putative candidate genes for the definition of berry quality traits.

## **Improving the annotation of unknown metabolites via integration of GCMS and LCMS metabolomics: A close look into the complexity of grapevine glycosides**

*Ghaste M., Narduzzi L., Carlin S., Vrhovsek U., Shulaev V., Mattivi F.*

Every grape cultivar has its own unique genetic characteristics, leading to the production of a different secondary metabolite profile. Volatile compounds in grapes are usually analyzed via GC-MS, while their glycosides are evaluated by indirect methods, after enzymatic or chemical hydrolysis.

A common feature of raw data from metabolomics experiments is that they cannot be immediately interpreted as relative concentrations of constituent compounds. A bottleneck here is the signal annotation, which is the process of tentatively associating the pseudospectra found with chemical structure. For the free volatiles, the annotation of large metabolomics dataset can be performed by an automatic pipeline that it is able to focus on hundreds of compounds simultaneously (Wehrens et al., 2014). However, there is scarcity of publications addressing the annotation of the glycosides, which makes the annotation of these compounds a challenging task. In this study we present the molecular profiling of volatile compounds and their precursors in ten selected genotypes, including *Vitis vinifera* cultivars, American species and interspecific crosses. We tried to “gain from the complexity”, since the difficult task of annotation of the glycosides was achieved through combined use of two orthogonal techniques, GC/MS and LC/HRMS, before and after enzymatic hydrolysis.

The results show that both free and glycosidically bound aroma precursors behave differently in each different grape cultivars and species. As many as 66 free aroma volatile molecules (originally existing and released after hydrolysis) were profiled through GC/MS analysis, while 15 glycosides were identified through LC/HRMS and correlation with GC/MS data.

**The high polyphenol content of *Vitis vinifera* cv. Tannat berries is conferred mostly by genes that are not shared with the reference genome**

*Da Silva C., Zamperin G., Ferrarini A., Minio A., Dal Molin A., Venturini L., Buson G., Tononi P., Avanzato C., Zago E., Boido E., Dellacassa E., Gaggero C., Pezzotti M., Carrau F., Delledonne M.*

The grapevine (*Vitis vinifera*) cultivar Tannat is cultivated mainly in Uruguay for the production of high-quality red wines.

Tannat berries have unusually high levels of polyphenolic compounds, producing wines with an intense purple color and remarkable antioxidant properties. We investigated the genetic basis of these important characteristics by sequencing the genome of the Uruguayan Tannat clone UY11 using Illumina technology, followed by a mixture of de novo assembly and iterative mapping onto the PN40024 reference genome. RNA sequencing data for genome reannotation were processed using a combination of reference-guided annotation and de novo transcript assembly, allowing 5901 previously unannotated or unassembled genes to be defined and resulting in the discovery of 1873 genes that were not shared with PN40024. Expression analysis showed that these cultivar-specific genes contributed substantially (up to 81.24%) to the overall expression of enzymes involved in the synthesis of phenolic and polyphenolic compounds that contribute to the unique characteristics of the Tannat berries. The characterization of the Tannat genome therefore indicated that the grapevine reference genome lacks many genes that appear to be relevant for the varietal phenotype.

## **Fertilization strategies to maximize the physiological grapevine performances**

*Tomasi D., Marcuzzo P., Petoumenou D., Gaiotti F.*

Since fertilization, with irrigation, is the main cultural practice that can drive plant development and balance, the meaning of fertilization strategies cover different aspects: i) type of nutrient, ii) delivery system, iii) quantity, iv) timing and v) aims. On this aspects our field experience proved that it is possible to manage the nutrients quantity, focusing on N and K, expecting different yield and quality results related to grape variety and enological objectives. In the meantime it is possible to enhanced the fertilization efficiency, taking into account the vine's nutrient demand along the growing season, timing of root uptake and the necessity to refill the nutrient reserves after harvest or during the maturation wood (end of the summer – eginning of the autumn). The nutrient delivery is part of the general strategies due to the fact that the vinegrower can apply above ground ertilization, sub soil fertigation, but also leaf application can solve some efficiencies (i.e. enhance yeast assimilable nitrogen YAN). Fertigation confirmed to increase yield and nutrient save, while controlled release fertilizers permit to have grape richer in color (anthocyanins). Considering plant the physiology, the goal is to manage nutrient to improve vine capacity reducing vine vigour. Autumn root reserves and starch accumulation are expected to create a better spring and summer vine balance. Three years of experiences carried out on Merlot variety confirmed a higher photosynthetic rate in controlled release fertilizer with positive effect on grape quality. Fertigation giving the opportunity to better balance the nutrients, permits to obtain grape with more aroma compounds (i.e. in pinot gris the quantity of 2 esenale was higher). Nowadays, sustainability is the main goal also in viticulture, and fertilization can act for this challenge avoiding luxury consumption, fertilizer leaching, reducing costs and giving the opportunity to recycle plant raw material (on farm compost obtained from winter pruning wood and pomace).

## **To store or not to store – what are the major postharvest challenges of table grapes?**

*Lichter A.*

Table grapes differ from wine grapes by numerous aspects but they share the same species so knowledge gained from each discipline can have mutual benefit. Wine grapes must be stored on the vines until they reach maximal Brix and color. For table grapes maximal ripening can be detrimental for storage and therefore means to reach optimal maturity in the vineyard were developed. These means include both enhancing ripening or delaying ripening by storage on the vines, making it possible to extend and control the ripening season. These means rely mainly but not only on plant growth regulators such as Ethylene and Absciscic acid to enhance color and Gibberellin and Cytokinin which were used so far to increase berry size. Storage on the vine also creates physiological and pathological risks which can be alleviated in several ways including shading and PGRs. To have better control over these vineyard manipulations, non-destructive monitoring tools were adopted which are able to measure the effect of the various tools. Harvest and storage technologies have not changed recently but novel 'shelf' technologies are available once current technologies will be abandoned. Both traditional and novel technologies can affect fruit flavor and one of the significant challenges is to point on the treatments that damage taste and on treatments that maintain or improve flavor. A central role in improvement of fruit flavor is reserved to new genetic resources which should stand up to all challenges including storage potential.



## **Comparison of spur and cane pruning in Shiraz and Cabernet sauvignon in northern Israel**

*Zahavi T., Harcavi E., Sapir G., Crane O., Nelevitzky R., Turgeman N., Lahat I., Shlissel M. and Liber I.*

Worldwide wine grapes are grown using either spur or cane pruning. Spur pruning is considered as the simpler method but in some varieties or under poor climatic conditions might result in low yield. The main drawback of cane pruning is in warmer regions, where bud burst along the cane is not even. The north part of the Golan is the coldest part of Israel, thus less bud break problems should occur. The aim in this study was to compare the yield and quality of grapes and wines made from the two pruning methods.

The experiment was conducted for three years in Fichman farm (900 m. altitude). Cabernet sauvignon (CS) and Shiraz (SY) vines were each planted in six rows, 2.75 m. between rows and one or 1.5 m. between vines in the row (in alternate rows). Half of each row was spur pruned and the other cane pruned to leave the same number of nodes for running meter. Vines were trained to VSP using two (CS) or three (SY) pairs of wires.

More shoots developed on spur pruned vines but the number of clusters/shoot was higher in the cane pruned vines so there was no difference in the number of clusters per vine or in total yield. Bud uniformity, calculated as average variance between shoot length on the cane was better in the spur pruned CS but in the cane pruned Sy. Light intensity in the cluster zone was measured in Sy vines at fruit set and was higher in cane pruned vines.

Micro-vinification was performed once on fruit from each variety in the experimental winery of Tel-Hai College. The differences in the CS wines were small and not significant while in Sy, the spur pruned vines yielded significantly preferred wines. Statistical differences were found in both tanins and antocyanins levels.

## **Spatial and diurnal below canopy evaporation in a desert vineyard**

Agam N., Kool D., Ben-Gal A., Lazarovitch N., Heitman J.L., Sauer T.J.

The architecture of wine-grape vineyards is characterized by tall plants and widely spaced rows. The wide row spacing is facilitating sunlight interception, air flow, and field operations. Consequently two distinct management zones can be identified: the vines and the inter-row. In arid regions, the inter-row is typically maintained with a bare soil surface, which acts as both a sink for solar radiation and a significant source of sensible heat, thereby affecting the energy and water exchange of the vines. Bare soil also allows direct soil water evaporation, a net loss of water that is unnecessary for grape production and is undesirable under water-limited conditions. The row structure and the typical application of drip irrigation result with significant differences in temperature and water content both in time and in space across the inter-row. These dynamics and their effect on the water and energy budgets of an isolated wine vineyard in the desert will be discussed.

## **Bacteria play go-between in insect-grapevine interactions**

Zchori-Fein E., Iasur-Kruh L., Sharon R., Zahavi T. and Naor V.

Bacteria from the genus *Phytoplasma* pose a major threat to many agricultural crops including grapevine. The conventional application of chemical sprays for controlling phytoplasma and other phloem restricted pathogens is inefficient, and the role of endophytes in the induction of plant resistance against such pathogens has been suggested. We hypothesized that host plants and insect vectors harbor microbes that affect phytoplasma infection. The current study is focused on the bacterial community composition of a planthopper (Hemiptera: Cixiidae) which serve as phytoplasma vector, as a source for such potentially beneficial bacteria. Sequences of 16S rRNA gene obtained from the planthopper showed dominance of *Sulcia* (77.95% of sequences in the sample), *Wolbachia* (4.51%) and a bacterium which belongs to Enterobacteriaceae (16.89%), with the remaining 0.65% of the sequences belonging to Actinobacteria, Alpha and Gamma-proteobacteria. Subsequently, bacteria were isolated from the insect on CV agar. One of these isolates belonged to the bacterial family Xanthomonadaceae, and its 16S rRNA sequence resembled one of the rare bacterial taxa found by the mass sequencing analysis. This isolate could be introduced via the roots to healthy and phytoplasma-infected grapevine plants, and its presence in plant tissues was confirmed by both fluorescence microscopy and molecular biology methods three weeks post inoculation. The effect of phytoplasma on plant morphology was markedly reduced in the presence of the isolate. Further study is needed to examine the potential use of this isolate as a bio-control agent against phytoplasma.

## **Grapevine response to arid environment: A systems approach**

*Hochberg U., Degu A., Lazarovitch N., Rachmilevitch S. Fait A.*

Deficit irrigation techniques are widely used in commercial vineyards. Nevertheless, varieties respond differently to water availability, prompting the need to elucidate the physiological and molecular mechanisms involved in the interactions between genotypes and their environment. Progressive water deficit was found to affect changes in leaf water potentials accompanied by metabolic changes in Shiraz and Cabernet Sauvignon. In both cultivars, but more intensively in Shiraz, water deficit caused a shift to higher osmolality and lower C/N ratios in the leaf, coupled with a highly coordinated change in the metabolic network. Correlation based network analysis also highlighted the structural role of major stress related metabolites, e.g., Pro, quercetin and ascorbate, which drastically altered their connectedness in the Shiraz network under water deficit. To study the mechanism of plant hydraulic adjustments, the cultivar petioles anatomy and hydraulic conductance was dissected. Results confirmed the link between petiole hydraulic architecture and hydraulic behavior providing a simple explanation for the higher transpiration rates commonly measured in Shiraz. Smaller xylem vessels in Cs could imply on its adaptation to WD, and explains its better performances under such conditions. In the same study, the variability in berry metabolism under deficit irrigation was investigated in the field. Berry skin metabolite profiling of the two cultivars was performed by parallel GC-MS and LC-MS at four development stages. In response to the water deficit CS maintained improved water balance, lower berry weight reduction and milder metabolic alteration of berry-skin primary metabolites, as compared with Shiraz. Polyphenol metabolism in response to water stress underwent significant changes, unique to each cultivar. The results are considered in the frame of the developmental regulation of metabolism of the two cultivars and suggest that tight hydraulic regulation can buffer the water-deficit driven changes in the berry skin metabolism with significant consequences on the metabolic composition of the fruit.

**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**

*Dequ A., Hochberg U., Sikron N., Venturini L., Buson G., Ghan R., Plaschkes I., Batushansky A., Chalifa-Caspi V., Fulvio M., Delledonne M., Pezzotti M., Rachmilevitch S., Cramer G.R. and Fait A.*

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 GC-MS and 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. In contrast, quite distinct pattern of change was apparent in specialized metabolites towards maturation, suggesting a varietal-dependent metabolic regulation. Transcript profiling revealed coordinated increased transcript abundance for genes encoding enzymes of committing steps in the phenylpropanoid pathway. The anthocyanin metabolite profile showed F3'5'H-mediated delphinidin-type anthocyanin enrichment in both varieties toward maturation, consistent with the transcript data, indicating that the F3'5'H-governed branching step dominates the anthocyanin profile at late berry development. The comparative metabolite profiles and RNAseq analysis of two physiologically different dark-skinned grape varieties revealed the underlying commonalities and cultivar-specificities of berry metabolism and its regulation. Enhanced stress related metabolism, e.g. trehalose, stilbene and ABA in Shiraz berry-skin corroborate its relatively higher susceptibility to environmental cues.

**Grapevine leafroll disease. Dynamics and damage in Israel**

*Nelevitzky R., Sapir G. Roshansky I. Zahavi T. and Crane O.*

Grapevine Leafroll Disease is one of the most widespread viral diseases of grapevine and is associated to the presence of closteroviridea. GLRaV1,3 are prevalent species in Israel, while number 3 is considered the most harmful one. In infected vines, the yield and quality of the fruit are damage. The loss of the quality is measured in low accumulation of sugar and in the berry color content, which lowers the quality of the wine produced. Symptoms of the disease are well known and include a change in leaf color (in red cultivars) and sometimes leaf roll. The goal of this work is to study the performance of the Grapevine Leafroll Disease and its effects on physiology and quality measures in the vines.

This study found a correlation between the date of the symptoms appearance and their symptoms severity level in harvest. During three years of research the abovementioned three vineyards showed that 5-10% of infected vines with symptoms did not display symptoms in consecutive years. Levels of sugar, pH and berry color were found opposite to the severity level of symptoms. It was also found that infected vines without symptoms similar in most measurements (Ripeness measures, berry size and potential water content) to healthy vines more then to infected vines with symptoms. By using qRT PCR it was found that difference in the titer of the virus between the infected vines without symptoms and infected vines with symptoms was only in the 'post' stage (in the beginning of the season).

## **Bacterial endophytes isolated from grapevines as a potential tool to reduce yellows disease symptoms**

Naor V., Barkai R. Bordolei R. and Zahavi T.

Yellows disease caused by phytoplasma is a major threat to vine growers worldwide. However, currently there is no efficient way to control the disease. The control of phytoplasma needs new strategies given that conventional application of chemical spray is inefficient. The role of endophytes in the induction of plant resistance against phloem restricted pathogens including phytoplasma has been suggested. However, in order to proceed towards practical application, such a candidate should be cultivable and able to penetrate and survive within the plant for a reasonable time. Bacterial endophytes were isolated from phytoplasma infected, recovered and healthy Cabernet-Sauvignon vines and from deserted vines. Out of 416 isolates 8 inhibited the growth of *Spiroplasma melliferum* as a model organism. Using Biolog and DNA sequencing of the 16S rDNA gene the endophytes were identified as bacilli. They were characterized based on colony! morphology formed on potato dextrose agar and by the molecular pattern formed using BOX-PCR analysis. The isolates were successfully re-introduced to grapevine and periwinkle plants by root dip and their presence was confirmed 7 d post inoculation. The presence of the bacteria did not affect the morphology of the plants. Further study is needed to examine the effect of these isolates on plants infected with yellows disease.

## **Boosting the synthesis of aromatic amino acids as precursors for the production of valuable polyphenol metabolites in grape fruit cell cultures**

*Manela N., Oliva M., Ovadia R., Sikron N., Fait A., Galili G. and Oren-Shamir M.*

Grapes (*Vitis vinifera*) are highly enriched with secondary metabolites derived from the aromatic amino acids, and mainly from phenylalanine. Within the phenylalanine derived metabolites, considerable attention focused on the health promoting activity of the phenolic compounds, stilbenoids and flavonols, which are considered the two major components of phyto-antioxidants responsible for the “French-Paradox” characteristics of the grapes. Here we present effective increase in the concentration of secondary metabolites in a grape cell suspension culture, using a recombinant feedback insensitive enzyme, under 35S promoter, derived from the bacterial AroG\* gene encoding the DAHPS (3-deoxy-D-arbino-heptulosonic acid 7-phosphate synthase) enzyme. DHAPS is responsible for the first enzymatic step in the conserved shikimate pathway leading to aromatic amino acid biosynthesis. Four transgenic lines transformed with AroG\* were established, and compared to two lines containing an empty vector. The AroG\* transgenic lines accumulated higher levels of phenylalanine and tyrosine, as well as health promoting secondary metabolites derived from phenylalanine such as resveratrol and dehydroquercetin. The main anthocyanin aglycons responsible for the red coloration of the cells, cyaniding and peonidin did not change significantly in concentration. However the minor aglycons, malvidin, petunidin and delphinidin decreased significantly.



## Mapping and characterization of *Vitis vinifera* ssp. *Sylvestris* in Israel

Brauner H., Drori E., Kahila G.

Wild grapevine is dioecious and characterized by high polymorphism in leaves. Male vine has large multiple blooms without style while the female has smaller blooms with degenerated pollen. To date there is no clear information about the distribution area of wild grape *V.v* ssp *Sylvestris* in Israel the diversity of the population whether it contributes or being donated genetically with cultured species *V.v* ssp *Sativa* in the area and if the local population of wild grapevine is similar or different from wild populations in other countries. The purposes of this study are: a mapping the distribution of wild grapevine in Israel; b morphologic and genetic characterization of individuals; c analysis of the population and comparison to European and Asian *Sativa* and *Sylvestris* populations; d examination of wine parameters of selected individuals. Preliminary results of this study indicate that the southern distribution limit of the wild grapevine is in the Kinneret lake region Sammak stream. An interesting phenomenon observed is a direct relationship between soil salinity and habitats of the wild grapevine. Genetic analysis among the collection of local wild species about 200 was performed by using 22 SSR. It seems that the local population of wild grapevine is significantly different from the population of the European wild grapevine. Results also show genetic relatedness between wild and local varieties. In terms of wine parameters, local wild grapes accumulate high levels of sugar 23-27 Brix, moderate pH 3.7-3.9 and high polyphenol content. Wine organoleptic properties would be analyzed in a few months.

## #6

### **Late season cluster thinning treatment**

*Drori E., Bar A., Green B.*

Cluster thinning, the removal of entire clusters from the vine following fruit set is a common practice which reduces yield and increases berry weight, soluble solids and color of table grapes. Research has been done on different dates of cluster thinning, ranging from fruit set, to veraison. Growers are concerned with the overall weight of their crop per acre. This figure changes dramatically throughout the season, from the second (lag) phase, through veraison and right up to harvest. We hypothesize that cluster thinning after veraison has a positive effect on yield - reducing berry size, increasing TSS and altering berry composition. We have conducted 4 treatments of cluster thinning, each being performed at different stages of fruit rippling dating from veraison to two weeks prior to harvest. Initial results from the first season of the experiment show that berry size has reduced, TSS content has grown and changes to fruit! composition may be evident.

**Cluster thinning**

Kitov A. & *Green B.*

Cluster thinning, the removal of entire clusters from the vine following fruit set is a common practice which reduces yield and increases berry weight, soluble solids and color of table grapes. Research has been done on the difference between the effect of variable timing of cluster thinning, ranging from fruit set, to veraison due to the concern of wine grape growers about the overall weight of their crop per acre. This figure changes dramatically throughout the season, from the second (lag) phase, through veraison and right up to harvest. We hypothesize that cluster thinning after veraison has a positive effect on yield - reducing berry size, increasing TSS and altering berry composition. We have conducted 4 treatments of cluster thinning, each performed at different stages of fruit rippling dating from veraison to two weeks prior to harvest. Initial results from the first season of the experiment show that berry size has been reduced, TSS content has grown and changes of fruit composition may be evident

**Sustainable nutrition management (Nutrigration & MultiCoTech)  
for Glera grapevine variety (*Prosecco wine*)**

Luison M., Biasi W., Bigot G., Schippa M.

We set up in 2013 a multi-year trial on Glera in the vineyards of Santa Margherita SpA in province of Venice; the purpose was to verify strategies to better manage the water and mineral nutrition through fertigation and granular fertilizer with controlled release nitrogen (MCT<sup>TM</sup>), in accordance with pre-established eonological objective, in a context of Sustainable Viticulture. In the vineyard were placed five different fertilization thesis: a granular traditional fertilization [fertilizer prompt action, N:K ratio of 1:1 for 80 kg/Ha of nitrogen units (thesis 1)], two theses with granular fertilization [ NPK+Mg based on controlled release nitrogen (MCT<sup>TM</sup>) applied at budburst/beginning of the growth], integrated with Fertigation (N:K ratio of 1:1 for Thesis 2 and N:K ratio 1:2 for Thesis 3, both with 80 kg/Ha of nitrogen units), two theses with only Fertigation (N:K ratio of 1:1 for thesis 4 and N:K ratio of 1:2 for Thesis 5, both with 60 kg/Ha of nitrogen units). The fertigation plans were made with 6 applications during the season, the source of potassium was potassium nitrate. Vine vegetative growth, NDVI test, yield and yield parameters (clusters/vine, cluster weight, berry size), grape macrostructure (soluble solids, titratable acidity, pH), yeast assimilable nitrogen (YAN) were determined. At the end of the first year we have seen that the thesis with only fertigation (thesis n°4 and n° 5) showed the best efficiency of fertilizer units UF applied (kg of grapes produced for UF applied); theses with fertigation + granular fertilizer (thesis n° 2 and n° 3) had a significantly higher yield with no worse grape quality parameters compared to the other theses; the panel test of the wines before sparkling process highlights how the thesis with fertigation+granular fertilizers (in particular thesis n° 3) have given better response.

## **Can we drive the wine style through the fertilization approach?**

*Marcuzzo P., Gaiotti F., Petoumenou D., Lovat L., Soligo S., Tomasi D.*

The consumer's demand for red wines in Veneto Region (North-East of Italy) has been changing in recent years, moving from traditional structured and alcoholic red wines towards fresh, fruity and low alcohol content red wines. A trial was carried out over the period 2011-2013 to set up optimum fertilization practices for two important red wine varieties cultivated in the Veneto Region (Merlot and Cabernet sauvignon), in order to guide the wine style toward the production of low-alcohol, fresh and aromatic red wines. Two commercial vineyards were selected in two different Provinces (Vicenza and Padova). In each site fertigation and/or NPK fertilization based on controlled release ( Multicote Agri ), were compared to the traditional local fertilization technique. The plans of fertigation and dosage of Multicote Agri were specifically designed in compliance with the oenological goals. Different irrigation systems (drippers and microjets) were tested for the fertigation; different application timings (spring and autumn) were tested for controlled release fertilizers. Yield and yield parameters (clusters/vine, cluster weight, berry size), grape macrostructure (soluble solids, titratable acidity, pH), anthocyanin and polyphenol contents, yeast assimilable nitrogen (YAN) were determined. At maturity, grape was harvested for small scale vinifications and the wines were subjected to sensory analysis. Results showed that both dripper and microjets fertigation increased the yield compared to the traditional fertilization, with no negative effects on grape quality. Both techniques were effective in reducing anthocyanin and polyphenols content in the musts, in prospect of producing fresher wines. Spring and autumn controlled release fertilizers increased the yield compared to that obtained with the traditional granular fertilization, but only the autumn application reduced sugar and anthocyanin contents. The wine sensory profiles highlighted important differences between treatments, indicating that it is possible to guide the wine styles toward specific oenological goals by using different fertilization approaches.

## #10

### **The fertigation of Pinot gris in friuli venezia giulia : three years of investigation with different rootstocks**

*Bigot G., Bigot L., Freccero A., Stecchina M., Schippa M., Sivilotti P.*

An experimental trial has been carried out in Friuli Venezia Giulia (North-Eastern Italy) during the years 2009-2012 on Pinot gris grafted onto different rootstocks, with the aim to compare traditional granular fertilisation with two different fertigation strategies in different rootstocks. A strip-plot design with 3 fertilisation treatments (granular fertilization, fertigation with low N/K rate and with high N/K rate) and 5 rootstocks (Kober 5BB, SO4, 110 R, 3309 C, 140 Ru) was set up and yield, grape nutrients and quality parameters were measured at harvest. The best economical results were obtained by the Kober 5BB rootstock. Both fertigation treatments provided higher yields and nitrogen in grapes, while quality parameters were slightly affected. Among rootstocks, 140 Ru resulted too vigorous for the site, while 110 R showed interesting performance.

## **Fertilization strategies to optimize the production and quality of grafted vines in Rauscedo area**

*Anaclerio F., Facchina S., Pavan G., Schippa M.*

In Rauscedo area (Friuli Venezia-Giulia) in 2006 started developing micro-irrigation ( drip-line ) and, as a result, fertigation. Experiments have revealed that fertigation leads to increased commercial grafted vines production ( larger presence of root systems in a radial pattern, higher content of starch, nutritional reserves as well as greater uniformity ), compared to the traditional fertilization technique. The trial was set up in 2013 to study the best base dressing fertilization (Pinot gris VCR5 / Kober 5BB VCR 102 with 140,000 plants/ha in double row). Three NPK granular fertilizers (containing different percentages and patterns of not prompt action nitrogen ) were tested . The same fertigation plane was applied in all plots. Two NPK granular fertilizer had different percentages of Controlled Release Nitrogen (CRN) with MCT coating [fertilizer of thesis n°1 had 35% of CRN and fertilizer of thesis n°2 had 47% of CRN]. The NPK fertilizer of thesis n°3 contained 25% of CRF (coating Poligen W3) and as 32% of total nitrogen inhibited with 3.4 DMPP. Theses n°1, n°2, and n°3 were tested against a control with no granular fertilization in base dressing but only fertigation (thesis n°4). The grafted vines pick up ( 3000 plants per thesis) were sorter according to commercial standards [ first-class (commercial grafted vines), second-class and waste]. Radical conformation (Roots opposite, Roots T and Roots radial) was also evaluated for a sub-sample of 200 first-class grafted vine (commercial). From the sorting is highlighted as the Thesis n°4 had a larger number of second-class and waste (14.7%) and a smaller number of first-class (commercial) (85.3%) compared to the other theses. Theses n°2 and n°3 yielded a larger number of Radial roots (67.5%, 68.0%), and a smaller percentage of Opposite and T Roots.

**New fertilization techniques to improve the nutrition efficiency**

Marcuzzo P., Gaiotti F., Petoumenou D., Lovat L., Soligo S., Tomasi D.

A three-year (2011-2013) trial was conducted to study the effects of two new fertilization techniques (fertigation and NPK fertilizers based on controlled release - Multicote Agri) on the production and quality of the Merlot variety in the AOC Piave area (North-East of Italy).

The objectives were to increase the overall grape and wine quality and improve at the same time the sustainability of the fertilization practice. In fact, these new fertilization techniques prevent useless nutrient leaching and improve the nutrient use efficiency (nutrients are provided to the plant according to its requirements), allowing for reduced application rates. Three treatments were compared: 1) Fertigation was applied 8 times between bud break and post-harvest, with 30N -15P - 81K units/Ha; 2) Multicote Agri was applied at the bud break stage, with 38N-15P- 80K units/Ha; 3) The traditional granular fertilizer (prompt action) was applied at the bud break stage with 38N-12P-80K units/Ha. Vine vegetative growth, yield and yield parameters (clusters/vine, cluster weight, berry size), grape macrostructure (soluble solids, titratable acidity, pH), anthocyanin and polyphenol contents, yeast assimilable nitrogen were determined. Moreover, grape was harvested for small scale vinifications and the wines obtained were subjected to sensory analysis. Results highlighted an effect of the fertilization practice on the vine growth and on the yield parameters, but the yield was comparable between the three treatments. Fertigation and Multicote Agri vines showed a more balanced canopy development compared to the traditional fertilization treatment vines. Concerning quality, Fertigation and Multicote Agri gave grapes with macrostructure similar to the traditional fertilization, but in both treatments the yeast assimilable nitrogen (YAN) in the musts was increased and in Multicote Agri anthocyanin and polyphenol contents were increased, too. Despite wine sensory profiles were strongly influenced by the year, wines from Fertigation and Multicote Agri obtained the highest scores for some important descriptors related to Merlot wine quality.



**Survey of Resveratrol levels in wines from different Israeli  
Terroirs, and development of agricultural methods to enhance  
Resveratrol synthesis in Grapevine**

*Stanevsky M., Cohen J., Nezer Y., Segev A., Shoseyov O. and Drori E.*

Resveratrol (RSV) is a stilbenoid molecule created in response to biotic and abiotic stress in grapevine. In the past two decades RSV has been proved to positively affect longevity, as well as effective in the treatment of modern diseases such as cancer, cardiovascular, oxidation and inflammation. This Survey was set out to evaluate RSV levels in 90 dry red wines from the 2012-2013 harvest in the diverse regions of Israel. In comparison to all the other wines Merlot RSV levels were significantly elevated in all the regions sampled. Furthermore, when comparing the effect of the region on RSV content, wines originating from the Golan-Heights and Samaria regions were significantly prominent than any of the other regions. Furthermore, Field trials to Examine the effect of defense response inducing plant hormones such as Methyl Jasmonate (MeJA) and Abscic Acid on RSV levels were set in cabernet sauvignon vinyard at Bravdo winery in Karmey Yosef, Israel. Initial results show that Vines treated by MeJA at verison show a fourfold (control – 0.6 ppm MeJA – 2.8 ppm) enhancement of RSV. All wines samples were analyzed using HPLC injection method. Maximum peak of trans-resveratrol was detected at 306-325 nm wave length. Berries were collected and frozen by liquid nitrogen. Sampels of berry skin were extracted with Acethyl acetate:Methanol (1:1) in the dark for 24H. After being centrifuged, supernatant was collected and dried by SpeedVac. The extracted samples were dissolved in methanol before HPLC injection.

## **Continuous measurement of soil evaporation in a drip-irrigated wine vineyard in a desert area**

*Kool D., Agam N., Lazarovitch N., Heitman J.L., Sauer T.J., Ben-Gal A.*

Vineyards have precise water requirements, making assessment of evaporation (E) relative to evapotranspiration (ET) particularly relevant. The lack of robust continuous and long-term measurement techniques to measure E is a critical problem for ET partitioning. In this study we assessed two novel techniques, the heat-pulse soil heat balance method (HP-SHB) method and a newly developed infrared thermometry (IRT) method, for continuous measurement of E in a drip irrigated vineyard in an arid environment. The HP-SHB successfully measured sub-surface E in the vineyard continuously over a season. As surface E dominates immediately following an irrigation event, high irrigation frequency limited the number of days where HP-SHB measurements were relevant. Good agreement was found between HP-SHB E and pre-season eddy covariance measurements as well as mid-season micro-lysimeter measurements. The high resolution data collection allowed assessment of diurnal patterns of E and the time at which E shifted to the sub-surface. The development of a method combining in-situ IRT measurements with micro-meteorological measurements below the canopy allowed fully continuous measurement for E, including periods before E reached the sub-surface. Similar to the HP-SHB method, a major advantage is that the method can be used without disturbing either the micro-climate or soil water fluxes. The infrared thermometry method was validated using both the data from a desert vineyard in the central Negev highlands, and from a temperate North Carolina vineyard. The results showed major potential for the IRT method as a continuous measurement of evaporation provided the below-canopy micro-climate can be adequately modeled.

## #15

### **Effect of different pruning treatments in Shiraz grape variety on wine composition and quality**

*Liber I., Lahat I., Shlisel M., Zahavi T. and Crane O.*

Wine quality is affected by the quality of grapes. The French term "terroir" Includes all the environmental factors that affect the quality of the vines. One of those factors is the microclimate around the bunch. Therefore, canopy structure has a major role in the quality of the berries. In this study we examined the effect of different pruning treatments on the resulting wine quality.

Shiraz vines were trimmed in two lengths : 1 m (short) and 1.5 m (long) leaving 2 buds and 8-14 buds, respectively. Three replicates were done for each pruning treatment. Other agriculture treatments were the same.

Wine was made from each replicate in the conventional procedure. During the grapes maturation and wine making some parameters as Ph, TA, VA, sugar content, Ethanol content, fermentation rate and malic acid concentration were followed. After six months the wine was bottled and the content of Tanins, Anthocyanins, and total phenolics compounds were analysed. A blind testing test was done with 12 experianced tasters.

The results indicate that there were no significant differences between the pruning treatments during grapes maturation. The fermentation rate of the long trimmed treatment berries was significantly faster than the short trimmed treatment. The Tannins contents as well as Anthocyanins, and total phenolics compounds was significantly higher in the short trimming treatment. The blind taste test results indicate that the short pruned treatment wine had significantly better grades than the long pruned wine. Our results suggests that short trimming treatment in the vineyard is better for wine quality.

## #16

### **Renewing oak barrel – Effect of toasting process on the recovery of volatile compounds**

*dvoskin Avidov A., shlisl M., Suskin G. and Popovich A.*

Alcoholic beverages are usually aged by storing them in oak barrels. The cost of the process is very high due to the high price of the barrels. The life span of the barrel is between 4-8 years. After this period of time, almost none of the volatile compounds are extracted to the wine.

We developed a control process for renewal oak barrels by abrasive 5 mm of the interior side of the wood and toasting with electrical heating element. The toasting process was examined for different temperatures (180,200,220 °c) and various time intervals (60,90,120 min). The concentrations of seven aroma compounds (vanillin, furfural, 5-Methyl Furfural, guaïacol , 4 Ethyl phenol, 2-Methoxy 4 Methyl Phenol, 4 Ethyl Guaiacol) were analyzed by GC-FID after extraction in Di chloro Methane (DCM) in contact with 1.5 gr of treated wood sawdust for 12 days.

We found that 4 of the volatile compounds ( , Guaiacol, 4 Ethylphenol, 2-Methoxy 4 Methyl Phenol, 4 Ethyl Guaiacol ) were not detected after the renewal process nor in the control.

Exposure the oak barrel wood to 220<sup>0</sup>c for 60 min. obtained the highest concentration of the other 3 volatile compounds e.g increasing of 15 fold for vanillin, 8 fold for furfural and 5 fold for 5-Methyl Furfural). Lower increase of these three volatile compounds was obtained in other temperature and time intervals exposure.

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