Schedule Overview

Date	Time	Session	Topic
Sunday August 1	6:00-9:00 PM	Welcome reception	(at Smith Opera House)
Monday August 2	8:30-8:40 AM	Opening Comments	
	8:40-10:05 AM	Oral Session 1	Pest and Disease Resistance I
	10:35-12:30 PM	Oral Session 2	Pest and Disease Resistance II
	12:30-1:30 PM	(lunch at Scandling Center)	
	1:30-3:00 PM	Poster Session 1	Berry quality; Adaptation to soils and climates; Genomics, transcriptomics, proteomics, and metabolomics; and Transgenic research
	3:00-5:40 PM	Oral Session 3	Grapevine Breeding: Techniques, Goals, and Strategies
Tuesday August 3	8:00-10:10 AM	Oral Session 4	Genomics, Transcriptomics, Proteomics and Metabolomics I
	10:40-12:35 PM	Oral Session 5	Genomics, Transcriptomics, Proteomics and Metabolomics II
	12:15-1:15 PM	(lunch at Scandling Center)	
	1:30-6:00 PM		Field Tour of NYS Agricultural Experiment Station, USDA-ARS units
	6:30 PM- ?		"Dinosaur Bar B Que" at Station Pavilion
Wednesday August 4	8:00-10:10 AM	Oral Session 6	Abiotic and Biotic Stress Tolerance
	10:40-12:35 PM	Oral Session 7	Genetics of Fruit Quality & Transgenic Research
	12:35-1:30 PM	(lunch at Scandling Center)	
	1:30-3:00 PM	Poster Session 2	Grapevine germplasm: Conservation and analysis; Breeding techniques, goals and strategies; Pest and disease resistance
	4:00 PM		Departure for evening banquet; winery stop en route
	6:00 PM		Arrival at Watkins Glen Harbor Hotel, reception, wine tasting
	7:00 PM		Dinner, entertainment by "Cool Club"
	10:00 PM		Return to Geneva
Thursday August 5	8:00-10:00 AM	Oral Session 8	Grapevine Genetic Resources - Parentage, Population Genetics, Fingerprinting
	10:30-12:00 PM	Oral Session 9	Breeding for Quality
	12:00-1:00 PM	(lunch at Scandling Center)	
	1:30 PM	Post conference tour	Tour to Keuka Lake vineyards and Hunt Country winery
	6:30 PM		Dinner at Chateau Esperanza
Friday August 6	10:00 AM	Post conference tour	Casa Larga Vineyards
			Drive through Lake Ontario tree fruit production region
			Continue to Niagara Falls
			Lunch at Hamlin Beach State Park, Lake Ontario

Saturday August 7		Post conference tour	Maid of the Mist boat ride to base of Niagara Falls
			Optional walk to the "Cave of the Winds"
	1:30 PM		depart Niagara Falls
			stop at Buffalo (2:30 PM) and Rochester Airports (4:00 PM)
	5:30 PM		Geneva

Welcome Reception at the Smith Opera House, 82 Seneca St., Geneva

Sunday, August 1, 2010

6:00 to 9:00 PM

(Wine, hot and cold Hors D'Oeuvres served)

Time	Presenter	Topic
7:00-7:45 PM	**Tom Burr,** Associate Dean, College of Agriculture and Life Sciences & Director, NYS Agricultural Experiment Station, Cornell University	Welcome on behalf of Cornell's College of Agriculture and Life Sciences and the NYS Agricultural Experiment Station
	Dariusz Swietlik, Area Director, North Atlantic Area, Agricultural Research Service, U.S. Department of Agriculture	Welcome on behalf of the U.S. Department of Agriculture - Agricultural Research Service
	Ben Ami Bravdo, Professor of Viticulture and Enology, The Hebrew University of Jerusalem & Chair, ISHS Section on Vine and Berry Fruits	Welcome on behalf of the International Society for Horticultural Science
	James Trezise, President, New York Wine & Grape Foundation	Welcome on behalf of the New York State Wine and Grape Industry
	Mayor Stu Einstein, City of Geneva, New York	Welcome on behalf of the City of Geneva, New York
	Bruce Reisch, Professor of Grapevine Breeding and Genetics, Cornell University & Convener of the 10th International Conference on Grapevine Breeding and Genetics	A brief history of grapevine breeding and genetics at the New York State Agricultural Experiment Station

Cornell University
College of Agriculture and Life Sciences
New York State Agricultural Experiment Station

ISHS UNIVERSITY of GUELPH USDA ARS

Monday August 2, 2010

Opening Plenary Session			**8:30-8:40 AM**
8:30-8:40		Welcome to the Symposium	Bruce Reisch, Peter Cousins

Oral Session 1: Pest and Disease Resistance I **8:40-10:05 AM**
Session Chair: Andy Walker, University of California, Davis, USA

Time	Oral Abstract Number	Title	Presenter
8:40-9:20	K-1	Keynote: Recent progress in understanding the genetics of pest and disease resistance in *Vitis*	Ian Dry
9:20-9:35	O-1	Genetic Mapping of *REN1*: A powdery mildew resistance gene present in two central Asian grapevines	C. Coleman
9:35-9:50	O-2	Additional homologs of the *Ren1* locus exist in Middle Eastern *Vitis vinifera*	S. Riaz
9:50-10:05	O-3	New generation of resistant table grape cultivars	S. Hoffmann
10:05-10:35		Coffee, Juice and Snack Break	

Oral Session 2: Pest and Disease Resistance II **10:35 AM-12:30 PM**
Session Chair: Jiang Lu, Florida A&M University, USA

Time	Oral Abstract Number	Title	Presenter
10:35-10:50	O-4	Introduction of application of Chinese wild *Vitis* species in China	Y. Wang
10:50-11:05	O-5	Identification of an R2R3 transcription factor involved in the regulation of the stilbene synthase pathway in grapevine	A. Vannozzi
11:05-11:20	O-6	Breeding for durable resistance to downy and powdery mildews in grapevine	D. Merdinoglu
11:20-11:35	O-7	Sequencing of the phylloxera resistance locus *Rdv1* of cultivar 'Börner'	L. Hausmann
11:35-11:50	O-8	Dissecting the genetic determinants of powdery mildew resistance in grape	E. Zyprian
11:50-12:05	O-9	Dissection of defense pathways in the grapevine-powdery mildew interaction by employing *Arabidopsis* defense-related mutants	W. Qiu
12:05-12:20	O-10	Development of molecular markers for powdery mildew resistance in grapevines	S. Mahanil
12:20-12:30		Summation of afternoon poster session	A. Nassuth, E. Rühl
(12:45-2:45 PM)		*(Grapevine CGC meeting; Creedon Room)*	(A.Walker, Chair)
12:30-1:30		Lunch at the Scandling Center	

Poster Session 1 **1:30 PM-3:00 PM**

Poster Number	Title	Presenter
Topic: Berry quality		
P-1	Genetic characterization based on the best linear unbiased predictor (BLUP) of traits related to the cluster architecture of *Vitis vinifera* L.	P. Hinrichsen
P-2	*PMEI* and *SPY:* Two genes with possible roles in seed and berry development in table grapes *(Vitis vinifera* L.)	X. Casanueva
P-3	Identification of genes related to stenospermocarpy in table grape *(Vitis vinifera* L.)	X. Casanueva
P-4	Gene-assisted selection for table grape breeding: Validation of a molecular marker for seedlessness	N. Mejia
P-5	Inheritance of some fruit quality characters in *Vitis vinifera* grapes	S.H. Li
P-6	Polyphenolic potential of the rare Croatian variety Dobricic (*Vitis vinifera* L.)	I. Budic-Leto
P-7	Impact of human selection on the allelic diversity of genes involved in the anthocyanin pathway	P. This
P-8	Monoterpene levels in different grapevine (*Vitis vinifera* L.) cultivars and clones during fruit ripening	M. Nitsch
P-9	A molecular marker system for the identification of the colour-locus in different tissues of grapevine (*Vitis vinifera* L.)	N. Ruh
P-10	Usefulness of the SCC8 SCAR marker linked to seedlessness in fungus resistant table grape breeding	T. Deak
P-11	Drawing links from transcriptome to metabolites: The evolution of Muscat aroma in the ripening berry	L. Costantini
P-12	The onset of berry ripening is characterized by hydrogen peroxide accumulation and lipid peroxidation	C. Moser
P-13	Ripening-related ethylene responsive factor characterization by *Agrobacterium*-mediated stable gene transfer in grapevine	A. Dal Ri
P-14	Morphological characteristics of cracking susceptible Korean table grapes, 'Heukgoosul' and 'Tamnara'	I.-C. Son
P-15	Characteristics of berry growth in tetraploid table grapes with different cracking susceptibilities in Korea	H.-K. Lim
P-16	A genetic analysis of berry quality traits in Spanish red wine grapes	C. Menendez
P-17	Genetic analysis of wine grape high-quality ripening on the Monastrell x Syrah progeny	A. Bayo-Canha
P-18	Use of molecular markers for seedlessness in grapevine breeding	M. Akkurt
P-19	The aroma-forming substances of hybrid seedlings of grape	A. Kataenko
P-20	Characterization of key components associated with the hormone signaling pathway that initiates berry ripening	L. Deluc
P-21	Anthocyanin profiling in the berry skins of five *Vitis amurensis* grapes and one related hybrid cultivar	F. He
P-22	Expression of early light inducible proteins (ELIPs) during the transition to autotrophy of *Vitis vinifera* L. leaves	D.E. Olivares
P-23	Changes of content and antioxidant activities of phenolic compounds during gibberellin-induced development of seedless muscat grapevine	T. Shu-fen

Topic: Adaptation to soils and climates

P-24	The development of molecular markers for *Vitis riparia* to use in interspecific breeding for freezing tolerance	A. Nassuth
P-25	Improving sustainable water use in grape production using a Drought Biomarker Detection (DBD) tool	S. Lund
P-26	Identification of ABA inducible genes from cold-hardy *Vitis amurensis* Rupr. Cv. Zuoshan-1 by suppression subtractive hybridization	Y. Zhang
P-27	Identification and applications of polyploidy in Muscadine grape (*Vitis rotundifolia* Michx.) breeding	X. Xu
P-28	In vitro selection of HYP tolerant cell lines from 'Chardonnay' embryogenic suspension culture	R. He
P-29	NCED expression and ABA concentrations vary with genotype in response to water deficit	D. Hopper

Topic: Genomics, transcriptomics, proteomics, and metabolomics

P-30	In silico SNP detection for genes of the anthocyanin metabolism in *Vitis*	V. Quecini
P-31	Grape berry development proteome: protein extraction and resolution by two-dimensional gel electrophoresis	P. Hinrichsen
P-32	*Vitis vinifera* genome annotation improvement using next-generation sequencing technologies and NCBI public data	P. Hinrichsen
P-33	Analysis of expressed sequence tags from *Vitis vinifera* flower and fruit and electronic mapping of new ESTs	J. Fang
P-34	Genome-wide identification of grapevine specific genes	Z.-M. Cheng
P-35	Evidence of a Ubiquitin Fusion Degradation 1 gene family in grapevine (*Vitis vinifera* L.)	L. Wei
P-36	Looking for genetic polymorphisms through clonal variation of Pinot noir	G. Carrier
P-37	SNiPlay, a web application for SNP analysis	P. This
P-38	Characterization of a new hormone in grape and analysis of its influence on grape development processes	A. Keren-Keiserman
P-39	Skin proteomic comparison among four grape cultivars with different anthocyanin contents	A.S. Negri
P-40	Towards a characterization of the PR-10 gene family in grapevine	A. Polverari
P-41	Assessing the genetic variability of grape clones	S. Vezzulli
P-42	Phenotypic plasticity in *Vitis vinifera*: how environment shapes wine	S. Dal Santo
P-43	Proteomic characterization of downy mildew-infected grapevine plants	A. Polverari
P-44	Transcriptomic characteristics of an Oriental cultivar of *Vitis vinifera*, Koshu	N. Goto-Yamamoto
P-45	Preliminary observations on the role of sirtuin genes in grapevine physiology	L. Bavaresco
P-46	Defense response to downy mildew in grapevine: Learning from transcriptomics and metabolomics	M.S. Pais
P-47	Grape berry cuticle under water deficits. Morphological, metabolomic and proteomic analysis	O. Zarrouk

P-48	Cioutat, a somatic variant from Chasselas exhibiting a programmed cell death-like phenotype	D. Lijavetzky
P-49	Characterization of grape *GAI* promoter in *Arabidopsis*	Y. Yang
P-50	Estimation of protein spot variability in 2-D gels	G. Cramer
P-51	Berry skin development of grape variety Norton exhibits distinct expression patterns of genes in defense and flavonoid pathways and unique profiles of flavonoid compounds	W. Qiu
Topic: Transgenic research		
P-52	Establishment of virus-resistance in grapevine rootstocks	G. Reustle
P-53	Marker-free transgenic grapevines using the Cre/lox recombination system	G. Buchholz
P-54	Towards a deep understanding of the function of grape flavonoid regulators VvMYB5a and VvMYB5b	M. Fasoli
P-55	*Agrobacterium rhizogenes*-mediated induction of transgenic hairy roots in *Vitis* species	Y. Jittayasothorn
P-56	Multifactorial analysis of the fungal tolerance in genetically modified grapevine plants: Defining prototypes for commercial purposes	P. Barba
P-57	Phenotypic evaluation of 'Thompson Seedless' grapes transformed with *AtNHX1* growing in hydroponics and potted soils	C. Aguero

2:30-3:00 PM	Coffee, Juice and Snack Break by Poster Session

Oral Session 3: Grapevine Breeding: Techniques, Goals, and Strategies **3:00-5:40 PM**
Session Chair: Peter Cousins, USDA-ARS Grape Genetics Research Unit, USA

Time	Oral Abstract Number	Title	Presenter
3:00-3:40 PM	K-2	Keynote: Progress in grapevine breeding	Rudolf Eibach
3:40-3:55	O-11	Recent trends and technologies for the improvement of seedless table grape breeding in Israel	A. Perl
3:55-4:10	O-12	Low-pressure selection for new grape crossings (Riesling italico x Pinot noir; Riesling italico x Chardonnay)	L. Bavaresco
4:10-4:25	O-13	Brazilian grape breeding program	P. Ritschel
4:25-4:40	O-14	Tetraploid table grape breeding in Japan	N. Mitani
4:40-4:55	O-15	Development of rootstocks for Australian conditions	B. Smith
4:55-5:10	O-16	Comparative cold hardiness of new cold climate cultivars and University of Minnesota breeding selections	P. Hemstad
5:10-5:25	O-17	The introduction, breeding, and extension of wine grape in China	L. Wang
5:25:5:40	O-18	Table grape breeding at the ARC INFRUITEC-Nietvoorbij, South Africa; Its impact on the SA industry and latest developments	P. Burger
6:00-8:00 PM		Dinner at the Scandling Center	

Tuesday August 3, 2010

Oral Session 4: Genomics, Transcriptomics, Proteomics and Metabolomics I 8:00-10:10 AM
Session Chair: Reinhard Töpfer, Institut für Rebenzüchtung Geilweilerhof, Germany

Time	Oral Abstract Number	Title	Presenter
8:00-8:40	K-3	Keynote: Grapevine genome update and beyond	Anne-Françoise Adam-Blondon
8:40-8:55	O-19	Integrated analysis of transcriptome and metabolome to understand grapevine development and its relation to wine attributes	H. Pena-Cortes
8:55-9:10	O-20	Deciphering the chromosome structure in *Vitis vinifera*: Genetic mapping, BAC-FISH and recombination	A. Canaguier
9:10-9:25	O-21	Identification and functional characterization of potential targets for genetic improvement of grape berry quality	S. Delrot
9:25-9:40	O-22	Identification of table grapes genes related to seedlessness, berry size and GA-responsiveness by functional genomics	P. Hinrichsen
9:40-9:55	O-23	Coordinate induction of anthocyanin biosynthetic pathway genes by *VvMybAs*	P.R. Poudel
9:55-10:10	O-24	Metagenomic sequencing as a tool to elucidate etiology - the case of the virome of a vineyard	J.T. Burger
10:10-10:40		Coffee, Juice and Snack Break	

Oral Session 5: Genomics, Transcriptomics, Proteomics and Metabolomics II 10:40 AM-12:35 PM
Session Chair: Gabriele Di Gaspero, University of Udine, Italy

Time	Oral Abstract Number	Title	Presenter
10:40-10:55	O-25	A gene expression map of *Vitis vinifera* cv. Corvina development	M. Pezzotti
10:55-11:10	O-26	Regulation of bud fruitfulness by floral meristem identity genes - Unique version of the *Arabidopsis* model?	E. Or
11:10-11:25	O-27	Transcriptional analysis of inflorescence and tendril development	J. Diaz-Riquelme
11:25:11:40	O-28	Haplotype structure and cluster phenotype associations for the *VvTFL1A* gene	L. Fernandez
11:40:11:55	O-29	Towards understanding the mechanisms of host resistance to downy mildew disease of grapevine by using genome wide expression profiling analysis	J. Lu
11:55-12:10		*(Announcements; information on Grapevine Phys. and Biotech. Meeting in Chile)*	
12:15-1:15		Lunch at Scandling Center	

Field Tour of New York State Agricultural Experiment Station, USDA-ARS units 1:30 PM till evening
Tour Leader:

Time	Topic
	Visit USDA-ARS Germplasm Repository, Grape Genetics Research, Grape Breeding Program, Vinification Lab, Viticulture Program
6:30 - ?	"**Dinosaur Bar B Que**" at the Experiment Station Pavilion <http://www.dinosaurbarbque.com/>

Wednesday August 4, 2010

Oral Session 6: Abiotic and Biotic Stress Tolerance **8:00-9:55 AM**
Session Chair: Annette Nassuth, University of Guelph, Canada

Time	Oral Abstract Number	Title	Presenter
8:00-8:40	K-4	Keynote: Genetics and genomics of grapevine responses to abiotic stress	Anne Fennell
8:40-8:55	O-30	Function of *Vitis* CBF transcription factors in stress tolerance	A. Nassuth
8:55-9:10	O-31	Mapping genetic loci for tolerance to iron induced chlorosis in grapevine *(Vitis vinifera* L.)	P.-F. Bert
9:10-9:25	O-32	Integrating ecophysiology and quantitative genetics to analyse the control of transpiration by the rootstock under drought conditions.	E. Marguerit
9:25-9:40	O-34	Cloning and characterization of the dagger nematode resistance gene, *XiR1*	C.-F. Hwang
9:40-9:55	O-35	QTL analysis of predatory mite abundance and leaf morphology traits in a hybrid grape population	C. Owens
9:55-10:25		Coffee, Juice and Snack Break	

Oral Session 7: Genetics of Fruit Quality & Transgenic Research **10:25 AM-12:20 PM**
Session Chair: Nami Goto-Yamamoto, National Research Institute of Brewing, Japan

Time	Oral Abstract Number	Title	Presenter
10:25-10:40	O-36	New insight into the genetics of color in grape	A. Fournier-Level
10:40-10:55	O-37	Differential expressed genes in berries of cv. Sangiovese (*Vitis vinifera* L.) during ripening following cluster thinning at véraison	C. Pastore
10:55-11:10	O-38	Co-Localization of QTLs for seedlessness and downy mildew resistance in grapevine	L.F. Revers
11:10-11:25	O-39	Polymorphisms in *VvPel* associate with variation for berry texture	J. Ibáñez
11:25:11:40	O-40	Quantitative analysis of texture and fertility in table grapes	I. Carreno
11:40:11:55	O-41	Gene silencing as strategy to induce grapevine fan leaf virus (GFLV) resistance in grapevine rootstocks	P. Arce-Johnson
11:55-12:10	O-42	Transcriptional changes in response to heterologous expression of a pear PGIP in grapevine	A.M. Ibáñez
12:10-12:20		Summation of afternoon poster session - P. This and R. Töpfer	
12:20-1:30		Lunch at Scandling Center	

Poster Session 2 **1:30 PM-3:00 PM**

Poster Number	Title	Presenter
Topic: Grapevine germplasm: Conservation and analysis		
P-58	Characterization of grape cultivars through ESTP	M.F. Moura
P-59	Phenotypic divergence among grapevine accessions of the germplasm collection at IAC	M.F. Moura
P-60	Brazilian Grape Germplasm Bank: Phenology and resistance to main fungal diseases	P. Ritschel
P-61	Grapevine variety determination from herbarium and archeological specimens	I. Pejic
P-62	Conservation, characterisation and management of grapevine genetic resources: The European GrapeGen06 project	P. This
P-63	Genetic structure and linkage disequilibrium in 4 *Vitis* species	A. Doligez
P-64	Genetic structure in a large representative sample of cultivated and wild grapevines	R. Bacilieri
P-65	Plastid DNA sequence diversity in a worldwide set of grapevine cultivars *(Vitis vinifera* L. subsp. *vinifera*)	T. Beridze
P-66	Analysis of grape rootstocks by microsatellite markers	G. Jahnke
P-67	Analysis of Pinot varieties by microsatellite markers	G. Jahnke
P-68	Italian wild grapevine: A state of the art on germplasm and conservation in 2010; the Year of Biodiversity	S. Imazio
P-69	Ribolla Gialla from northeastern Italy, Rebula from northern Balkans and Robola from Ionian islands: do they belong to the same population variety or are they genetically different?	S. Imazio
P-70	Proteomic analysis among different Aglianico ecotypes	A.S. Negri
P-71	Viticultural performance of some Cabernet Sauvignon clones	L. Bavaresco
P-72	The software for a 'Universal Grapevine Database'	C. D'Onofrio
P-73	Molecular characterization of the grapevine germplasm collection held at the Fondazione Edmund Mach	S. Grando
P-74	Application of SSR markers for must and wine varietal detection	M. Baleiras-Couto
P-75	Variation of berry polyphenolic profiles of USDA *Vitis vinifera* germplasm	Z. Liang
P-76	Aestivales (Planchon), American native grapes: Phylogenetics and use for breeding of high quality wine grapes for southeastern United States	V. Colova
P-77	Morphological and molecular identification of Maragheh grapevine cultivars	Y. Sharafi
P-78	Genetic diversity in Maragheh grapevine pollen traits	Y. Sharafi
Topic: Breeding techniques, goals, and strategies		
P-79	Studies on an excellent early-mature bud sport of 'Kyoho'	L. Wei

P-80	Generation of a new polyploid grape cultivar by a new strategy that combines crossbreeding with colchicine-induced mutation	D. Zhigang
P-81	Effectiveness of different culture media on the success of embryo rescue among different crosses of seedless grapes	Y. Hao
P-82	Polyploid induction of mutation by using colchicine on tube seedlings of Victoria grape	J.-L. Zhu
P-83	Some early maturing table grape cultivars with muscat flavor released by the Beijing Institute of Forestry and Pomology	L. Sun
P-84	Progress in grapevine breeding in China over a decade	J.-L. Zhu
P-85	New triploid seedless table grape cultivar 'Moonlight Seedless' bred in China	S.J. Zhao
P-86	Conferred vigour: Loci but no logic	S. Decroocq
P-87	Grape breeding and viticulture research program in Iran	J. Mahmoudzadeh
P-88	'Tankeumchu': A mid-ripening, black-fruited table grape	J. Noh
P-89	The usefulness of allotetraploidy and isolated embryo culture in vitro for obtaining intergeneric hybrids of grape	A. Kataenko
P-90	Genetic control of phyllotaxy phase shift in juvenile vines in a rootstock hybrid population	P. Cousins
P-91	Drying rate of fresh berries from natural dry-on-the-vine (DOV) grape germplasm.	D. Ramming
P-92	Quality improvement in Vignoles through clonal selection	P. Cousins
P-93	Development of table and raisin grapes with high anthocyanins using a leaf disk assay.	D. Ramming

Topic: Pest and disease resistance

P-94	Mechanisms of powdery mildew resistance in the Vitaceae family	A. Feechan
P-95	Reaction of grape rootstocks to *Meloidogyne incognita* and *M. javanica*	M.F. Moura
P-96	Global gene response to viral infections in grapevine	M.C. Medina
P-97	Genetic analysis of the resistance to foliar diseases on *Muscadinia rotundifolia*	S. Blanc
P-98	Investigation of the interaction of *Elsinoë ampelina* with *Vitis vinifera*	A. Kono
P-99	Berry cracking caused by powdery mildew (*Uncinula necator*) infection to 'Fujiminori' grapevines *(Vitis* sp.)	H.-S. Shin
P-100	Gray Mold *(Botrytis cinerea)* induced berry cracking in 'Fujiminori' table grapes (*Vitis* sp.)	D. Kim
P-101	Elicitation of grapevine defense responses against downy mildew caused by *Plasmopara viticola*	M. Selim
P-102	Optimizing the breeding of Pierce's disease resistant winegrapes with marker-assisted selection	A. Walker
P-103	The biological characteristics of *Xylella fastidiosa* in xylem fluid from resistant and susceptible grapevines	X. Shi
P-104	Development of marker-assisted selection as a tool for breeding disease resistant grapes	B. Reisch

P-105	Pathogen responsiveness and variety/tissue specificity of five stilbene synthase genes in grapevine	R. Dai
P-106	Characterization of grapevine *NPR1*	Y. Zhang
P-107	Screening grape hybrid families with molecular markers linked to resistance genes	E. Kiss
P-108	The genetic mapping of *Xiphinema index* resistance derived from *Vitis arizonica* by using SSR markers	S. Van Zyl
P-109	Characterization of an ankyrin-like protein up-stream region from *Vitis vinifera* is highly induced by *Botrytis cinerea* infection	P. Barba
P-111	Cloning and functional characterisation of a putative powdery mildew susceptibility gene in grapevine	I. Dry
P-112	Evaluation of grapevine germplasm from La Rioja for tolerance to powdery mildew (*Erisiphe necator*)	C. Menendez

Poster session additions

P-113	Analysis of genetic structure of twelve Sicilian grapevine cultivars	O. Failla
P-114	Evaluation of 4 new rootstock genotypes obtained by back cross	O. Failla
P-115	Genetic transformation of grape (*Vitis vinifera*) to increase salt tolerance	A. Zurita-Silva

2:30-3:00 PM	Coffee, Juice and Snack Break by Poster Session

4:00 PM	Departure for evening banquet at the Harbor Hotel, Watkins Glen, with winery stop en route
6:00 PM	Arrival at Harbor Hotel, reception, wine tasting
7:00 PM	Dinner, entertainment by "Cool Club"
10:00 PM	Return to Geneva

Thursday August 5, 2010

Oral Session 8: Grapevine Genetic Resources - Parentage, Population Genetics, Fingerprinting
Session Chair: Patrice This, INRA, Montpellier, France **8:00-10:00 AM**

Time	Oral Abstract Number	Title	Presenter
8:00-8:15	O-43	Genetic characterization of the USDA grape germplasm collection: Challenges and lessons learned	S. Myles
8:15-8:30	O-44	The Turkish grape *(Vitis vinifera* L.) SSR database	A. Ergul
8:30-8:45	O-45	Genealogy investigation in over 2300 grapevine cultivars *(Vitis vinifera* L.)	T. Lacombe
8:45-9:00	O-46	Association genetics in relation with berry size variation in grapevine (*Vitis vinifera*)	C. Houel
9:00-9:15	O-47	Intravarietal variability of Crljenak Kastelanski and its relationship with Zinfandel and Primitivo selections	I. Pejic
9:15-9:30	O-48	Discrimination of grapevine clones by methylation sensitive AFLP analysis	G. Reustle
9:30-9:45	O-49	Molecular survey of Georgian grapevine genetic resources	S. Imazio
9:45-10:00	O-50	Allelic variation in the domestication gene *VvmyA1* in natural grape populations *(Vitis vinifera* ssp. *sylvestris*)	R. Arroyo-Garcia
10:00-10:30		Coffee, Juice and Snack Break	

Oral Session 9: Breeding for Quality **10:30-12:00 Noon**
Session Chair: Enrico Peterlunger, University of Udine, Italy

Time	Oral Abstract Number	Title	Presenter
10:30-10:45	O-51	Marker-assisted breeding: Identification of monoterpensynthases in grapevine (*Vitis vinifera* L.) and their potential as markers in breeding	O. Bitz
10:45-11:00	O-52	Functional characterization of terpene synthases of aromatic and non-aromatic grapevine varieties	C. D'Onofrio
11:00-11:15	O-53	An integrated approach to studying the positional candidate gene 1-deoxyxylulose-5-phosphate synthase (*VvDXS*) involved in Muscat flavor	F. Emanuelli
11:15-11:30	O-54	The study of triploid progenies crossed between diploid and tetraploid grapes	L. Sun
11:30-11:45	O-55	Characteristics of promising Muscadine grape (*Vitis rotundifolia* Michx.) selections from the University of Georgia (U.S.A.) breeding program	P. Conner
11:45-12:00		Business meeting: Venue for meeting in 2014	B. Bravdo
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1:30 PM Departure of Post Conference Tour

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K-1

Recent progress in understanding the genetics of pest and disease resistance in *Vitis*

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Grapevines are highly susceptible to a wide range of pests and microbial pathogens including insects, nematodes, fungi, oomycetes, bacteria, phytoplasmas, viruses and viroids. Historically, grape growers have relied heavily on the use of pesticides and fungicides, in combination with various management techniques, to minimise the impact of these pathogens. There is, however, increasing financial, regulatory and market pressure on grape growers to minimize the application of agrochemicals in the vineyard. An example of this is the recent proposal by the European Commission to ban the use by 2013 of a large number of agrochemicals in Europe which are routinely used for the control of grapevine fungal diseases. In the face of these increasing pressures, the development of new grapevine cultivars with improved "natural" genetic resistance to pathogens is a high priority. This review will summarize our current understanding of the genetic basis of resistance to plant pathogens and outline a number of different molecular strategies currently being used to develop pathogen resistant grapevine germplasm either by genetic transformation or marker-assisted breeding. This includes not only the mapping and cloning of dominant resistance genes from wild grapevine species but also the potential application of strategies which target "susceptibility" genes which predispose plants to infection by certain pathogens.

K-2

Progress in grapevine breeding

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Since the introduction of phylloxera and the mildew diseases from North America to Europe during the second part of the 19th century, grape breeders around the world have been engaged in introducing into the gene pool of the European quality vines resistance traits existing in wild American and Asian species. Meanwhile these activities have led to remarkable success, resulting in cultivars with high wine quality and a high degree of resistance against downy mildew as well as powdery mildew, allowing a considerable reduction of plant protection. Nevertheless it can be stated that breeding progress in the past has had considerable limitations. Apart from the long generation cycle, the extensive evaluation necessary for a range of complex traits as well as the time-, labour- and space consuming cultivation of grapes are major restrictions. As a consequence, knowledge about the genetics and the inheritance of viticulturally important traits like resistance or quality characteristics was poor. However, substantial progress on these aspects has been achieved in recent years. Various research groups have generated genetic maps for various genetic backgrounds which allowed the identification of different loci carrying genes for crucial traits, especially resistance traits. The establishment of marker assisted selection (MAS) as a new tool for grapevine breeders offers plentiful possibilities for increasing breeding efficiency. It allows not only monitoring the segregation pattern of these resistance loci in the progeny but also identifying the genotypes with multiple resistance loci. This pyramiding by MAS should lead to a higher and an increased sustainability of the resistances. Similarly MAS allows a targeted selection of parents which have the potential for pyramiding resistance loci in their progeny. The establishment of elite breeding lines with homozygous resistance loci is another option which can only be realized by MAS. From such parents all offspring carry the resistance loci. Finally, MAS allows an accelerated introgression of desired traits from wild species into the gene pool of *Vitis vinifera*. The use of marker assisted selection for the target locus combined with a background selection for a high percentage of *Vitis vinifera* genome speeds up this process considerably. Summing up, it can be stated that the described newly available breeding tools and strategies will mark a paradigm shift from empirical to knowledge-based breeding.

K-3

Grapevine genome update and beyond

A-F. Adam-Blondon*[1] and the French-Italian Public Consortium for the Sequencing of the Grapevine Nuclear Genome[2]

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The grapevine (*Vitis vinifera* L.*)* is the most economically valuable fruit crop in the world. Its haploid genome is estimated to be about 500 Mb, organized in 19 chromosomes. The grapevine is the fourth flowering plant whose genome sequence has been made public. A French-Italian public consortium project has recently completed a 12X Whole Genome Shotgun sequence of a quasi-homozygous genotype, PN40024. All data were generated by paired-end sequencing plasmid, fosmid and BAC libraries of different insert sizes, using Sanger technology. Using 11.91X coverage, an assembly of 499 Mb was obtained, composed of 2888 super-contigs, 91% of which are anchored on linkage groups. Different approaches revealed that approximately 41% of the grape genome is of repetitive/transposable elements (TE) origin. The automatic annotation led to an estimate of 26,347 protein coding gene models. The grape genome was shaped by two ancient whole genome duplications that were not followed by extensive rearrangements, thus enabling the discovery of ancestral traits and features of the genetic organization of flowering plants. This sequence enables powerful integrative approaches for the identification of key genes for important traits in grapevine and offers the potential to completely renew strategies for its breeding.

K-4

Genetics and Genomics of Grapevine Responses to Abiotic Stress

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The Vitaceae family is adapted to diverse environmental conditions as evidenced by the distribution of species in temperate and subtropical climatic regions. However, production of quality grapes for wine, juice, raisin and table grapes is frequently limited by abiotic stress as a result of limited cultivar selection and/or marginal environmental conditions for growth and development. The ability to identify, develop and match genotypes to geographical regions is also limited by the complexity of abiotic stress (drought, salt, and temperature) traits, scion/rootstock interactions and the long generation time in grapevines. Although many common responses have been identified for abiotic stressors, identification of tolerant phenotypes coupled with genetic and genomic analysis is critical for defining the genetic mechanisms that promote optimal timing responses resulting in tolerance to a given abiotic stress. Phenotypic and genetic analysis coupled with emerging genomic information and tools provide exciting opportunities to improve selection for and development of management strategies for abiotic stress tolerance.

O-1

Genetic mapping of *REN1*: a powdery mildew resistance gene present in two central Asian grapevines

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Powdery mildew disease is one of the most widespread fungal diseases of grapevine, causing damage to the fruit and foliage of susceptible vines. All of the grapevines of the species *Vitis vinifera* were thought to be susceptible to this pathogen, but recently two varieties of *V. vinifera* grapes from Central Asia were found to be resistant to the powdery mildew fungus. The resistance phenotype is due to the presence of a single dominant gene named *REN1* (*Resistance to Erysiphe necator 1*) present in both varieties. A relationship study was performed on these two resistant grapevines, 'Kishmish vatkana' and 'Dzhandzhal kara', which revealed that they can be related as closely as half-siblings. Genetic mapping of the *REN1* locus was performed using existing and newly designed microsatellite markers on 461 plants in two mapping populations. *REN1* was mapped to a genetic distance of 1.4 cM between markers sc47_14 and sc47_20 on chromosome 13, which corresponds to a physical distance of 1.4 million bases on the PN40024 reference sequence. This enabled an analysis of the gene content of the *REN1* homologous region in PN40024, which is characterized by the presence of partial and complete NBS-LRR genes. Markers flanking the *REN1* interval can be used in breeding for marker assisted selection of resistant plants and markers within the interval can be used to test new recombinants for further restriction and analysis of the region containing *REN1*.

O-2

Additional homologs of the *Ren1* locus exist in Middle Eastern *Vitis vinifera*

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The discovery of the powdery mildew resistance locus, *Ren1*, in two Central Asian *Vitis vinifera* cultivars, 'Kishmish vatkana' and 'Dzhandzhal', was an important contribution to powdery mildew resistance breeding. Allelic information on the SSR markers flanking this locus enabled the screening of additional germplasm in search of other homologs carrying powdery mildew resistant genes. This study screened 368 accessions of table and wine grape varieties that originated from the Middle East, Afghanistan, Turkmenistan, Pakistan and Central Asia, as well as Chinese *Vitis* species and hybrid direct producers of Native American *Vitis* species origin. In the first round of tests, six international standard SSR markers were used on the complete set of accessions to identify synonyms and homonyms. In the second round, ten additional markers sourced primarily from chromosome 12, 13 and 18 were utilized on a set of 263 unique accessions. The allelic diversity of marker VMCNg4e10.1 and UDV124 that flank the *Ren1* locus was investigated. Thirty-one accessions were identified that had the 216 bp allele for marker UDV124 and 30 accessions had the 260 bp allele of marker VMCNg4e10.1, both of which have been associated with *Ren1*. The six accessions with resistant alleles for both markers included two cultivars with known resistance to powdery mildew, 'Kishmish vatkana' and 'Karadzhandal' (a synonym for 'Dzhandzhal'), and four other cultivars 'Husseine', 'Late Vavilov', 'Sochal', and 'Black Kishmish'. All of the accessions with one or both alleles linked to resistance were screened under field conditions conducive to powdery mildew infection. Preliminary results indicate that the four additional homologs of *Ren1* have significant resistance to powdery mildew.

O-3

New generation of resistant table grape cultivars

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The whole table grape market and producers worldwide are missing high quality combined with downy, powdery mildew and botrytis resistant table grape cultivars. Breeding of resistant table grape cultivars is even more difficult than breeding of resistant wine grape cultivars, due to the quickly growing quality demands (perfect seedlessness, large berry size, crunchiness, etc.) and the lack of complex resistance sources for table grapes. In grapevine resistance breeding programs mostly wine grape cultivars were used for backcrossing wild resistance sources, so again some new backcross generations need to be generated to transform the wine grape attributes into table grape attributes. Our resistance breeding strategy is based on the pyramiding of resistance genes of different origin against one pathogen to gain durable resistance and prohibit pathogens to break through the resistance. The aim is to select genotypes with pyramided resistance against both mildews and get a high level of botrytis tolerance and storability, besides reaching the highest quality demands. Of course, it can be performed in more generation cycles, and requires marker assisted selection. The monogenic, eventually oligogenic inheritance of resistance genes is also a prerequisite, because during the following generations of backcrosses for resistance gene pyramiding and quality advancement, loci of polygenic resistance can not be followed by markers and would be lost. Accordingly, in our breeding program the RUN1 gene from *Muscadinia rotundifolia* is supported by the REN1 gene from *Vitis vinifera* cv. Kishmish vatkana. The downy mildew resistance is inherited by Rpv1 gene from *Muscadinia rotundifolia* and supported by Rpv4 gene from *Vitis amurensis*. Botrytis resistance is based on morphological characters inherited from significantly tolerant cultivars. Some of our most important segregating populations contain the following attributes: Rpv4 + seedlessness, earlyness, excellent quality; REN1 + Rpv4 + seedlessness; RUN1 + REN1 + Rpv1 + seedlessness. Starting from wine grape characters, in each generation there is big advancement in table grape quality attributes. The latest populations are already at the third generation diverging from wine grape, and are hopefully ready for the selection of the first resistant and seedless table grape cultivar candidates.

O-4

Introduction and application of Chinese wild *Vitis* species in China

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China is one of major centers of origin of the *Vitis* species. Chinese wild Vitis species are naturally distributed in the majority of areas throughout China, but mainly distributed in Huazhong Area and Qinba Mountain Area located in Shaanxi and Gansu provinces in China. To date, more than 35 species and varieties have been reported with origins in China among the approximately 70 known species. In the past 20 years, Researchers have focused on the study of the resistance to diseases *Uncinula necator, Plasmopara viticola, Sphaceloma ampelinum, Coniathyrium diplodiella and Glomerella cingulata* of *Vitis* species or varieties native to China by means of field identification, field inoculation and laboratory isolated inoculation. The results indicated that the species and clones indigenous to China are resistant or highly resistant to the diseases. There are differences in resistance to the diseases among all these species and clones. However, the Chinese wild *V. pseudoreticulata* accession "Baihe-35-1" hold higher resistance to disease traits than the other wild species. Cold hardiness of 17 wild *Vitis* species native to China, including 38 clones, was assessed by growth, tissue browning and electrolytic conductance methods. The results showed that the cold hardiness of *V. amurensis* and *V. yeshanensis* were highest as compared to other species. Chinese *Vitis* species have many advantages over *V. vinifera* such as possessing many resistant genes to main fungi diseases as well as good flavor. Especially *V. quinquangularis* and *V. amurensis* clones hold better economical traits than the other wild species and many disease resistant genes. So, they are used as wine materials directly in southwest and northeast areas in China; and they exhibited high resistance to fungal diseases, they were also easier to hybridize with *V. vinifera*. Thus, it is possible to use crossing to integrate fungal disease-resistant traits from the wild Chinese *Vitis* into *V. vinifera*, thus resulting in fungal disease-resistant new grape varieties. At present, we are interested in studying how Chinese wild grapes sense and respond to fungal diseases using *V. pseudoreticulata* accession Baihe-35-1 as material. Particularly, we are interested in understanding regulation of disease resistance signaling mechanisms and disease resistance genes under *Uncinula necator* stress in Chinese wild *Vitis* species. Present research involves analysis of function of target genes using bio-chemical method *in vitro*, and also genetic and molecular characterization of Arabidopsis mutants that are altered in response to the pathogen stimuli. The goals are to elucidate the

Chinese wild grape signaling pathways in response to the pathogen stress and to identify key components that can potentially be engineered for improvement of European grapes to encounter pathogens stresses. The other objective of our study was to develop an efficient method for *in vitro* embryo rescue and plant development for the hybridization of *V. vinifera* (seedless) as female parents with wild Chinese *Vitis* (fungal disease-resistant) as male parents. With this protocol, hybrids from 11 cross combinations using Chinese wild *Vitis* containing fungal disease-resistant genes as male parents were successfully obtained. Evaluation of these regenerated plants on resistance to fungal diseases is under investigation. In the future, our goals areto understand the host defense mechanism and identifyi key genes in resistant Chinese wild *Vitis* germplasm and to provide valuable information and foundational resources for the timely and efficient molecular breeding of highly resistant cultivars using a combination conventional hybrid breeding and bio-technology methods.

O-5

Identification of an R2R3 MYB transcription factor involved in the regulation of the stilbene synthase pathway in grapevine

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Stilbene synthases (STSs) are a class of enzymes belonging to the general CHS type III polyketide synthase family involved in the last step of the biosynthesis of stilbenes. These enzymes and their main products (resveratrol or pynosylvin) are detectable in only a limited number of unrelated plant species, including grape, and accumulate in response to biotic and abiotic stresses. Despite numerous studies that have been performed on the accumulation, metabolism and biological properties of resveratrol, little is known about the transcriptional regulation of this pathway. Based on microarray data obtained from grape cell cultures treated with jasmonic acid we identified a candidate R2R3 MYB transcription factor that shows an expression pattern similar to that observed for STSs and could to be involved in their regulation in grape. This R2R3 MYB factor was designated *VvMYB14*, based on homology with the *AtMYB14* R2R3 MYB factor. Neither gene has previously been functionally characterized in either plant species. Analysis of *VvMYB14* expression in grape leaf discs treated with biotic (downy mildew infection) and abiotic stresses (wounding and UV-C exposure) known to be involved in the transcriptional activation of *STS* genes, showed a close correlation between the pattern and timing of expression of selected *STS* genes and *VvMYB14*. Using a Dual Luciferase Reporter Assay System in transiently transformed grapevine cells, VvMYB14 was also demonstrated to increase stilbene synthase promoter activity. Confirmation of the role of *VvMYB14* in the transactivation of VvSTS genes *in planta* is currently being examined using a transgenic grapevine hairy root system for testing the effect of both silencing and overexpression of *VvMYB14* on the response of VvSTS expression. Preliminary results indicate that roots in which VvMYB14 has been silenced, show significantly reduced levels of *VvSTS* transcription following the application of an abiotic stress. Further experiments are now underway to clarify the role of VvMYB14 in the regulation of both the stilbene synthase pathway and genes belonging to the general phenylpropanoid pathway in grapevine.

O-6

Breeding for durable resistance to downy and powdery mildews in grapevine

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A wide range of pathogens threatens viticulture. The current strategy to control grapevine diseases relies totally on the use of fungicides. This practice not only is expensive but also causes a slow and progressive damage to the environment. A cost-effective and environment friendly alternative to the use of chemicals is the development of varieties resistant to pathogens. All traditional European grapevine varieties are susceptible to the main pathogens responsible to the chemical treatments. However *Vitis* species closely related to cultivated grapevine were already shown to be potential sources of resistance to a wide spectrum of grapevine diseases. The absence of private grapevine breeders in France led the INRA to design a breeding program dedicated to create new resistant varieties. The main goal of this programme is to create varieties durably resistant to downy and powdery mildews with a berry quality suitable to produce high quality wines. In order to successfully reach the double objective of high resistance efficiency and durability, the use of multiple sources of resistance was planned as soon as the project was designed. The project was developped in close interconnection with upstream research programmes which mainly aimed at understanding the genetic bases of the resistance to downy mildew derived from grapevine-related wild species by addressing four key questions: exploring the diversity available in genetic resources to chose original genitors; identifying and characterising the relevant genes/QTLs to genetically improve the targeted traits, using the data acquired on genes/QTLs (position, effects) to assist the selection with markers, and assessing the durability of the identified resistance genes/QTLs. Moreover the data from the programmes carried out on the determinism of resistance to powdery mildew, berry quality components, sex and phenology are progressively integrated.

O-7

Sequencing of the phylloxera resistance locus *Rdv*1 of cultivar 'Börner'

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In the middle of the 19th century phylloxera (*Daktulosphaira vitifoliae* Fitch) was introduced from North America to Europe and dramatically damaged the viticulture. The insect attacks the root tips and older roots of grapevine leading to gall-like deformed structures called nodosities and tuberosities, respectively. The former gall type results in slight damage but the latter type leads together with secondary fungal infections to the dieback of the whole vine. To overcome the problem phylloxera-tolerant rootstocks were developed from American wild *Vitis* species onto which scions of European grapevine cultivars were grafted. Nevertheless most rootstock cultivars currently used show nodositiy symptoms after phylloxera infection. In contrast, rootstock cultivar 'Börner', an interspecific cross of *V. riparia* Gm 183 and *V. cinerea* Arnold, is phylloxera-resistant as it shows neither tuberosity nor nodosity symptoms. To characterize the genetic basis of the 'Börner' phylloxera resistance a segregating population derived from the cross V3125 (Schiava grossa x Riesling) x 'Börner' was used to establish a genetic map. The artificially infected progeny was phenotyped concerning nodosities and a single quantitative trait locus, *Rdv*1, could be localized on chromosome 13. Fine mapping was performed developing synteny-derived STS markers deduced from the PN40024 reference sequence. Further resolution was obtained with a local map based on a set of recombinant vines restricting the *Rdv*1 locus down to a size of about 350 kb related to the reference sequence. From a pooled BAC library clones were isolated covering the entire region and subsequently sequenced. A small RGA cluster is located in the *Rdv*1 locus as it is the case in the corresponding region of the reference sequence. However, size and structural arrangement of the repetitive resistance genes differs clearly giving reason to identify candidate genes for phylloxera resistance within this core region.

O-8

Dissecting the genetic determinants of powdery mildew resistance in grape

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Grapevine (*Vitis vinifera* L.) in European viticulture is threatened by pathogens like powdery and downy mildew, requiring heavy fungicide applications to ensure good harvests. Concerns about environmental impacts necessitates the development more sustainable production methods. New, high quality cultivars with genetic resistance are therefore generated. To this purpose resistance traits from American and Asian *Vitis* wild species resistance carriers are introgressed into the cultivated susceptible *V. vinifera*. However this breeding is a long term process, requiring many years of plant propagation and phenotypic evaluation in the progeny of controlled crosses. Molecular markers linked to traits of interest and knowledge about the underlying molecular mechanisms help to accelerate this long term breeding, rationalizing the choice of parentals and replacing part of the phenotypic evaluation by genetic testing at early stages of plant growth. Molecular markers linked to downy and powdery mildew resistance have been identified by genetic mapping and quantitative trait analysis in the past. In a recent complementary study, differential gene expression was investigated under powdery mildew challenge as compared to non-challenged plants of resistant (`Regent´) and susceptible (`Chardonnay´) grapes grown axenically *in vitro*. A set of 27 genes was then selected from a long list of differentially regulated transcripts detected by microarray hybridizations and studied in more detail by quantitative Real Time PCR. Results confirm an early and strong induction of some transcription factor genes whose products are instrumental for the reprogramming of the cellular transcriptome during early defense reactions. The sequence diversity of their genes and additional key regulators of defense signalling cascades has been investigated in a set of 45 genotypes with varied levels of powdery mildew resistance. A high number of single nucleotide polymorphisms have been found and analysed for their implications on encoded amino acids or potentially regulatory sequence motifs. More than 100 SNPs identified in six genes were subjected to association analysis with powdery mildew resistance expression. Among those, 14 turned out to be strong candidates and are currently being analysed in a larger sample set of 350 grapevine accessions including various sources of resistance. Results from this work will be presented.

O-9

Dissection of defense pathways in the grapevine-powdery mildew interaction by employing *Arabidopsis* defense-related mutants

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The molecular interactions between grapevine and the obligate biotrophic fungus *Erysiphe necator* have not been understood in depth because of the recalcitrance of grapevine to genetic modifications. Defense-related mutants have been available for *Arabidopsis thaliana*, which is an excellent model for elucidating molecular mechanisms underlying host-pathogen interactions. Arabidopsis mutants that are susceptible to pathogens provide valuable genetic tools for grapevine geneticists to identify key components in perception, signal transduction and regulatory circuits in the mounting of defense responses in grapevine. We have employed these mutants to verify the functions of salicylic acid (SA)-mediated defense genes including *Enhanced Disease Susceptibility1* (*EDS1*) of grapevine, and to characterize the functionality of promoters in response to SA and *E. necator*. In addition, we are using the Arabidopsis *npr1-1* mutant to confirm the regulatory role of grapevine NPR1 in the activation of defense-related genes. With these results, we begin to get a glimpse of molecular events that determine disease resistance levels in *Vitis* species native to the North American continent. Our results demonstrate the utility of Arabidopsis genetic resources in understanding the genetic mechanism of grapevine disease resistance. Currently we are applying these mutants in the genetic dissection of grapevine defense components and in verifying the roles of defense-related genes that have been discovered by functional genomics analysis of grapevine-pathogen interactions.

O-10

Development of molecular markers for powdery mildew resistance in grapevines

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Molecular markers have been used as a tool for plant breeding for two decades. Markers linked to disease resistance can be used to pyramid resistance genes for improved durability and to improve the efficiency of evaluation. To develop molecular markers linked to resistance to grape powdery mildew (*Erysiphe necator*), *Vitis romanetti,* a source of resistance from China, was used to create a mapping population. A pseudobackcross 2 population was produced by crossing C87-41 [(*V. romanetti x V. vinifera*) x *V. vinifera*] with B70-57 (*V. vinifera*). Resistance was assessed in multiple locations and years by both natural and artificial inoculation. A genetic map was constructed using one CAP, 127 SSRs and 27 SNPlex markers. This source of disease resistance is qualitative, fits a single-resistance gene model and was mapped to linkage group 18. A screen of eighteen offspring with a 9,000 SNP Infinium genotyping microarray identified 20 SNPs (across 3.4 Mb) on linkage group 18 that are tightly linked with the resistance locus. Together these markers will be a valuable tool for marker assisted selection of powdery mildew resistance.

O-11

Recent trends and technologies for the improvement of seedless table grape breeding in Israel

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Seedless grapes are generally preferred by consumers for fresh consumption. Approximately 80% of the table grapes exported in the Western world are seedless. The general objectives in most breeding programs of table grapes can be summarized as follows: improved eating quality extension of the season by creating early maturing types and late maturing types, seedless selections lacking seed rudiments under diverse growth conditions, attractive appearance, large berry size, bright colors, unusual shapes, long shelf life, firm flesh, naturally loose bunches, uniform berries, and novel tastes and aromas. Most commercial seedless cultivars are stenospermocarpic and require normal fertilization for fruit set. Plant growth regulators are one of the important factors affecting the success of embryo rescue and have been widely used to improve the efficiency of embryo germination during the *in vitro* stage. The effect of growth regulators applied in the vineyard, before and after anthesis, on the efficiency of embryo rescue will be discussed. Application of several phytohormones enabled the temporary conversion of seedless maternal lines lacking any rudiment into seeded, thus increasing the choice of maternal lines suitable for cross pollination. Examples for different, new and promising selections recently developed in our breeding project will be presented. These selections represent different breeding goals and are targeted to different sectors of the fresh grape market.

O-12

Low-pressure selection for new grape crossings ('Riesling italico' x 'Pinot noir' and 'Riesling italico' x 'Chardonnay')

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A breeding programme by intra-specific crossing was set up in 1989, in the Experimental Station "Riccagioia" (Lombardia region, northwest Italy), with the aim of getting new varieties mainly for white sparkling wine. *V. vinifera* L. 'Riesling italico' was used as mother, while 'Pinot noir' and 'Chardonnay' were used as fathers, resulting in two progenies (RPN and RCH). 400 and 900 seedlings were obtained from RPN and RCH crossings respectively, and a low-pressure field selection was performed, choosing 66 individuals out of the 1300, by utilizing as screening criteria vegetative habit, phenology, ripening, health aspects (especially viruses, Esca disease, excoriose, yellows diseases). The selected seedlings were grafted on SO4 rootstock, and the productive and qualitative grape parameters of the field-growing vines were checked over a 4-year-period (2002-2005) by multifactorial analysis of variance (MANOVA) and SNK (Student Newman Keuls). DNA fingerprinting (by microsatellites) of the progenies and the parent varieties was performed as well, confirming the parentage. Grapes were vinified at harvest, and the wines were evaluated according to sensory analysis, by processing the main descriptors by the non parametric Friedman test. According to the above mentioned parameters, three new varieties were selected, as follows: RCH10 and RPN33 (both white berry) and RPN26 (red berry), due to a good canopy (RCH10), high acidity and sugar and very low cluster and berry weight (RPN26). RCH10 and RPN33 are interesting for sparkling wine because of their white color, the wider sensorial profile and the highest pleasure indices, as compared to some of the parents ('Riesling italico' and 'Pinot noir'). RPN 26 has an unexpected later ripening and a wine color intensity much higher than 'Pinot noir', being suitable for the production of red wines; under high-pressure selection tightly connected with the objectives of this breeding program, this genotype would have been excluded in the first step of the selection procedure.

O-13

Brazilian Grape Breeding Program

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In Brazil, the first records of genetic grape breeding are dated from XIX century, but only after 1940 the developmentof grape breeding programs started at public institutions. Since 1977, Embrapa Grape and Wine has been leading a breeding program, aiming to get grape cultivars for different purposes as table grapes, grapes to make wine and juice and also the developing of rootstocks. The objectives of this program evolved together with Brazilian viticulture and can be resumed as the developing of grape cultivars adapted to different production regions, including tropical ones, presenting tolerance to the main Brazilian grape diseases and plagues and quality to different purposes. About 3000 hybrids from crossings between several *Vitis* species, including wild tropical ones, are evaluated each year. Selected individuals are multiplied and evaluated for 3-4 years. Then, promising materials are propagated and evaluated in greater plots for more 3-4 years. This step can include sensory analysis of table grapes, juice or microvinifications, depending on the grape selection purpose. Advanced selections are then tested on commercial fields, for about 2 years. New cultivars are released only when this decision is also supported by growers. As results, 14 new Brazilian grape cultivars were released in recentyears, contributing to the several segments of grape productive chain. ‘D. Zilá’ and ‘Tardia de Caxias’ are table grapes of ‘labrusca’ type, while ‘BRS Morena’; ‘BRS Clara’ and ‘BRS Linda’ are seedless table grapes of ‘vinifera’ type adapted to tropical climates. ‘Moscato Embrapa’ and ‘BRS Lorena’ are wine grapes with muscat flavor which can be recommended to tropical areas. ‘BRS Margot’ is a hybrid producing a ‘vinifera’ type wine. ‘BRS Rúbea’, ‘BRS Cora’, ‘BRS Violeta’ and ‘BRS Carmem’ are juice grapes with very strong color and high sugar content. ‘Concord Clone 30’ and ‘Isabel Precoce’ are early variants of traditional grape cultivars of ‘labrusca’ type, Concord and Isabel respectively. Currently, about 300 table and processing grape selections are under evaluation and five new advanced selections are under validation. These point toward the perspective of development and release of new Brazilian grapes in the next years.

O-14

Tetraploid Table Grape Breeding in Japan

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A table grape breeding program has been carried out at the National Institute of Fruit Tree Science (NIFTS) since 1968. The main objective of this program is to release new excellent cultivars adapted to Japanese climatic conditions. In addition, large berry size, high eating quality (especially firm and crisp flesh), and no berry cracking have been the principal considerations. Furthermore, new cultivars should be converted to be seedless easily with gibberellic acid (GA) treatment because seedless berries are a desirable commodity for consumers. For the improvement of berry size, tetraploid cultivars/selections have been used as cross parents and five tetraploid cultivars for table use have been released from NIFTS until 2009. ‘Aki Queen’ resulted from the selfing of ‘Kyoho’. The fruit of ‘Aki Queen’ ripens a little earlier than ‘Kyoho’. Its berries are converted to be seedless and weigh 13g with two GA treatments and bright red in color. ‘Sunny Rouge’ resulted from the cross between ‘Pione’ and ‘Red Pearl’ and its fruit ripens earlier than ‘Kyoho’ and later than ‘Delaware’. Its berries are converted to be seedless and weigh 6g with two GA treatments and red-brown to violet red in color. In GA non-treated condition, the bunch is not well filled due to berry shattering. ‘Dark Ridge’ resulted from the cross between ‘Kyoho’ and 301-1 (‘Kyoho’ × ‘Niabell’) and the ripening time is the same as ‘Kyoho’. Its berries are well-black and colored more easily than those of ‘Kyoho’ even in warm regions in Japan, and they weigh 12g in GA non-treated condition. ‘Honey Venus’ resulted from the cross between ‘Benizuiho’ and ‘Olympia’ and the ripening time is the same as ‘Kyoho’. The flavor seems to be a mixed one of muscat and foxy. Its berries weigh 10g in GA non-treated condition. Recently, seedless fruits of ‘Honey Venus’ have begun to be produced in several regions in Japan. Flesh of these five cultivars is firmer and crispier than that of ‘Kyoho’, and berries of these cultivars, apart from ‘Sunny Rouge’, are larger than 10g. At present, enhancing fungal pathogen resistance is also under investigation.

O-15

Development of rootstocks for Australian conditions

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Soils in some major Australian viticultural regions are generally high in potassium content, which is in contrast to viticultural regions in other parts of the world, where potassium may be sufficient or deficient. There is a considerable body of evidence, both from commercial experiences and research results, that the widely adopted high vigour, nematode tolerant rootstock varieties contribute to negative impacts on wine quality associated with high potassium uptake, high pH and malate levels, which require tartaric acid supplements in winemaking for pH adjustment, and reduced colour in berries and poor spectral properties in red wine. Grapevine rootstocks provide a key vineyard management tool to address issues of high soil potassium levels, as well as soil borne pests, (nematodes and phylloxera), abiotic stresses (e.g. those attributed to drought and soil salinity), vine vigour and yield, and fruit composition. Through the use of both glasshouse and field screening assays, advanced selections of rootstocks are being identified for potential commercial application specific to Australian viticulture. Previous findings have demonstrated that *Vitis cinerea* is resistant to the root-knot nematode *Meloidogyne incognita*. We have determined that it also possesses resistance to *Meloidogyne javanica*. Lengthy field trials of rootstocks have been analysed for fruit and juice traits of the scion. The potassium content of ungrafted vines is highly correlated with the pH of resultant grafted plants. Early selection of potential new rootstocks is possible based on the petiolar potassium content and root-knot nematode resistance of young vines. Genotypes which meet the criteria of low potassium accumulation and root-knot nematode resistance are being evaluated for phylloxera tolerance, good rooting of dormant canes, high graft compatibility with fruiting scions, appropriate vigour, and drought tolerance.

O-16

Comparative cold hardiness of new cold climate grape cultivars and University of Minnesota breeding selections

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The University of Minnesota has been actively engaged in breeding cold hardy and disease resistant wine grape cultivars since the late 1970s. Since that time, four new cultivars have been released that are now widely grown throughout the northern states of the U.S. and parts of Canada. In conjunction with this ongoing effort, germplasm is routinely screened for primary and secondary bud survival under very harsh local winter conditions. The low temperatures for the winters of 2008-2009 and 2009-2010 were -32°C and -28°C, respectively. In the spring of 2009, well ripened, medium diameter canes were collected from 17 named cultivars and 22 breeding selections. Buds were then dissected and examined under a microscope for oxidative browning. The same procedure was used in the spring of 2010 to analyze the bud survival of 20 cultivars and 24 breeding selections. The highest levels of survival in 2009 were seen with the cultivars 'Marechal Foch' and 'Sabrevois' and the breeding selections MN 1178 and MN 1258. The poorest survival was seen with 'Castel 19637' and MN 1247. In 2010, five genotypes had 100% primary bud survival, including 'Frontenac gris', St. Croix', MN 1198, MN 1204, and MN 1271. The lowest bud survival was seen with the same two genotypes as the previous year, 'Castel 19637' and MN 1247. These laboratory dissection results will be compared with data from observed bud survival in the vineyard.

O-17

The introduction, breeding and extension of wine grape in China

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The earliest viticulture in China was traced to Han Dynasty more than 2000 years ago. Modern viticulture only began in the 1890s' with the birth of Zhangyu Grape Wine Company which introduced 120 cultivars to test in China. These cultivars belong to *Vitis vinifera* such as 'Cabernet Sauvignon' , 'Merlot', 'Chardonnay' and 'Reisling'. However, the viticulture including wine grape had very small area (3200 ha) before 1949. The wine grape breeding programs in China were initiated from *V. vinifera* × *V. amurensis* in the Institute of Botany, Chinese Academy of Sciences in 1954. The other research units also introduced western and eastern European cultivars to evaluate and select at the same time. Although there was a little increase of viticulture land in the 1950s', the extension of wine grape was slow until the early 1970's. With wine grape 'Beichun' released by Institute of Botany, Chinese Academy of Sciences, 'Beichun' became a main wine-making cultivar in the south of China with 7000 ha expanding in area from 1970s'-1980s'. *V. vinifera* such as 'Cabernet Sauvignon' became a main variety for wine from 1990's to now. However, 'Beihong' and "Beimei' released by the Institute of Botany, CAS were extended rapidly in the past 5 years and produce the strong competition to 'Cabernet Sauvignon' et.al because these two cultivars have higher tolerance to cold and fungus and 24-27% soluble solid content. In addition, wine grape 'Gongniang 1' and "Gongniang 2' from *V. vinifera* × *V.* amurensis were introduced by Jilin Academy of Agricultural Sciences, and three 'Shuang' series cultivars were originated from *V.* amurensis in Chinese Academy of Agricultural Sciences. These cultivars had also high cold tolerance, but no good wine characters, therefore they were only grown in Northeast of China. 'Yan 73' and 'Yan 74' which are good cultivars for drying were introduced by Zhangyu Grape Wine Company. The 3 'Mei' series grape cultivars for red wine and 2 'Quan' series grape cultivars for white wine were released by Shandong Vine and Wine-making Institute. However, these cultivars were spread only in small areas and basically kept as grape germplasm materials.

O-18

Table grape breeding at the ARC Infruitec-Nietvoorbij, South Africa: its impact on the South African industry and latest developments

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Since its inception in 1952 and first releases in 1965, the ARC table grape breeding programme released a total of 28 cultivars. In 1985 one ARC cultivar (Bien Donné) made a noteworthy contribution to export (3%), while the contribution of six ARC cultivars was 25.9% of the total export in 2006. Changes in consumer preferences necessitate regular revision of aims. For example, export of seedless cultivars from South Africa grew from a minimal contribution in 1985 to over 52% in 2006 and is still growing. New seedless cultivars are developed by *in vitro* embryo rescue techniques, while crosses to develop seeded cultivars with unique characteristics are also made. The first seedless cultivar was released in 1986, followed by another five seedless cultivars. Two new, early ripening, red seedless cultivars will be released in near future to the South African industry. Since 2004, DNA technologies and molecular approaches were introduced as an essential component of the disease resistance breeding program. An extensive DNA fingerprint of table grape cultivars/selections was constructed and is used to solve identity issues. 'Regent', an interspecies hybrid, is resistant against powdery and downy mildew (Fisher *et al* 2004) and was used to introduce this resistance into the ARC table grape breeding program. We report validation of the resistance loci previously identified, following a QTL approach in a Regent x Red Globe population. We identified plants that phenotypically displayed resistance to both downy and powdery mildew, whilst also carrying flanking markers for both resistance QTL. These plants are prime candidates to be used in future crosses to introduce resistance, whilst also selecting for good fruit quality. The population and the genetic map is a valuable resource that can be used in future to map other disease resistance loci present in Regent or fruit quality traits of RedGlobe.

O-19

Integrated analysis of transcriptome and metabolome to understand grapevine development and its relation to wine attributes

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Different physiological and biochemical processes are involved in fruit setting, development and ripening of grapevine berries. Diverse efforts using varied technologies are being applied in order to explain certain key biological stages of this fruit which could allow, in a near future, to improve the quality of grapevine berries and consequently the quality of wine. To gain information concerning the genes and metabolites involved in such processes, we measured primary and secondary metabolites together with transcript levels of three *Vitis vinifera* cultivar berries during their growth period. Samples of cultivars Carmenère, Merlot and Cabernet Sauvignon were collected every three days and during two growth seasons, starting with flowers and finishing with mature berries. We established a qRT-PCR platform to investigate the transcriptional changes during growth development and setting that allows us to determine the expression levels of around 800 grapevine genes. We measured metabolite levels by using GC-TOF-MS and UPLC/FT-MS for primary and secondary metabolites respectively. Since huge amount of data is generated, we use a network analysis approach based on correlations as a tool to investigate the relationship between transcripts and metabolite changes and their role in regulation of metabolic pathways during ripening. Understanding the processes involved in grape ripening will contribute to obtain better fruits quality which would help to improve wine quality. Considering the advantages provided by using LC-MS to analyse secondary metabolites, we are applying this technology to investigate whether a non-targeted wine chemical composition analysis could provide suitable information on the relationship between the quality scores -given to Chilean commercial red wines by experienced winemakers- and wine matabolome data and in that way provide an objective method to discriminate wine quality scores and other wine attributes like variety, origin or vintage. The analysis of the metabolic profiles of the wine samples by unsupervised multivariate techniques and *in house* developed bioinformatics tools recognize signatures"(Biomarkers") in wine metabolome which allow to differentiate attributes like variety, vintage, origin and wine quality scores.

The knowledge acquired will allow to identify potential biomarker of quality present in the fruit and the wine and will also allow to determine the correlation among them. Such kind of information can be used to improve agricultural techniques in order to obtain better quality fruits for better wines.

O-20

Deciphering the chromosome structure in *Vitis vinifera*: genetic mapping, BAC-FISH and recombination

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Within the framework of the sequencing project for the grapevine genome (Jaillon et al 2007), two genetic maps were completed with SSR markers in order to align the sequence contigs along the chromosomes. Thus, two full sib populations, 'Syrah' x 'Grenache' (SYxGR, 192 individuals; Doligez et al. 2006) and 'Chardonnay' x 'Bianca' (CHxBI, 358 individuals; Doligez et al. 2006) on which, respectively, 223 and 401 markers have been used. These two maps allowed anchoring over 90% of the reference genome sequence (Cipriani et al. in prep.). Recently, SNP markers were defined from 38 anchored but not oriented super-contigs of sequence and 44 super-contigs not oriented and not anchored. So far 110 new markers were mapped, allowing anchoring of 21 not yet anchored super-contigs (~3.2Mb) and orienting 32 super-contigs. In depth analysis is ongoing. In parallel, the recombination rate has been analyzed along the chromosomes for each of the four parents using the first version of the two maps with the software TETRA (Drouaud et al 2006). This software associates a probability with the observation of hot spots or cold spots of recombination. Thus, the recombination rates have been compared with the density of repeats sequences, genes, and GC%. Moreover, we are developing a cytogenetic map in *Vitis vinifera* using BAC-FISH. All these approaches will be combined to give a pattern of the *Vitis vinifera* genome, including the factors affecting its recombination rates and the dynamics of the genome evolution.

O-21

Identification and functional characterization of potential targets for genetic improvement of grape berry quality

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Grape berry and wine quality and typicity depend on fruit metabolite content (sugars, organic acids, flavonoids, aroma precursors). This content results from complex interactions between two genotypes (rootstock and variety) and the environment. Genetic selection requires identification of genome regions and genes which control the metabolism and accumulation of these compounds. Our lab has developed several strategies that may lead to this identification. Based on previous data with model plants, targeted approaches using PCR and a wide range of functional genomic tools (transcriptomics, transient and stable transformation) led us to identify several transcription factors (belonging to Myb and Wky families) and regulatory proteins (protein kinases) involved in either the control of sugar transport or secondary metabolism. *VvSK1,* a GSK3 protein kinase, is strongly expressed at post-véraison, when the berries accumulate glucose, fructose, and abscisic acid (ABA). In grapevine cell suspensions, *VvSK1* transcript abundance is increased by sugars and ABA. *VvSK1* overexpression in grapevine cells increased the transcript abundance of four monosaccharide transporters. At the same time, the rate of glucose uptake was increased three to five fold and the amount of glucose and sucrose in the cells was doubled, while the starch amount was not affected. Overexpression of VvWKY2 in tobacco induces drastic modifications in lignin composition, especially the Syringyl/Guaiacyl ratio, in xylem vessel diameter, and alters the organization of the vascular structure. Transient expression in tobacco protoplasts showed that VvWRKY2 activates the promoter of the grapevine *C4H*. Overexpression of *VvMYB5b* in tomato induced pleiotropic changes including dwarfism, modified leaf structure, alterations of floral morphology, pigmented and glossy fruits at the “green-mature” stage, and impaired seed germination. *VvMYB5b* overexpression downregulated the phenylpropanoid pathway whereas carotenoid metabolism was upregulated. The strongest modification was a decrease in beta-amyrin, the precursor of the oleanolic acid, which is the major component of grape waxes. VvMYC1, a member of the bHlH family, was characterized as a component of the transcriptional complexes controlling anthocyanin and proanthocyanidin biosynthesis during the development of grape berries. Non-targeted approaches are now developed to identify regulatory genes controlling the response of berry metabolism to changes in the microenvironment (light, temperature). These approaches and results will be illustrated.

O-22

Identification of table grapes genes related to seedlessness, berry size and GA-responsiveness by functional genomics

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A table grape breeding program was initiated 15 years ago by the Chilean Institute of Agriculture Research, INIA. This Program is now part of the 'Biofrutales Consortium', a private-public partnership. The breeding approach of the Program has been to increasingly incorporate the use of biotechnological tools to improve the efficiency and precision with which parents and segregants are selected. The first step in this approach was to build a framework linkage map based on ca. 120 co-dominant SSR markers and the subsequent identification of QTLs for traits such as seedlessness and berry size. Later on, physical, genetic and *in silico* maps were integrated and about 20 candidate genes were identified. Within the major QTL for seedlessness and berry size, the *AGL-11* gene was located on LG18 and found responsible for a large fraction of the variance for both traits. Two other candidate genes, *SPY* and *PMEI*, were associated to a secondary QTLs and characterized by RT-PCR in different genetic backgrounds. Details of this association will be presented on a separate paper. Currently, segregants from a 'Ruby Seedless' x 'Thompson Seedless' cross, which exhibit contrasting phenotypes for seed and berry size, are being used to study the transcriptome of berries at different stages of development (fruit set, 2-4 mm ∅ and 6-8 mm ∅), treated or not with gibberellic acid. This population is under analysis by hybridization (Combimatrix™) or by massive cDNA sequencing (Illumina™). Also the proteome is being analyzed in 2-D gels associated to mass spectrometry. Those genes consistently detected by two or more of these approaches, will be individually screened by RT-PCR to determine their expression level. Also, a position on the reference map will be proposed by looking for co-locations of these genes and the secondary QTLs. The possible association between the phenotypic expression and the allelic variation will be analysed in search of markers that could be used as a selection tool in INIA's Table Grape Breeding Program.

O-23

Coordinate induction of anthocyanin biosynthetic pathway genes by VvMybAs

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Anthocyanins are the major flavonoid of grapes and are responsible for the development of red to black color in grape berry skins. Anthocyanin biosynthesis occurs through the phenylpropanoid and flavonoid pathways. Recent studies in grapevine revealed that VvMybA transcription factors control the expression from UDP-glucose flavonoid 3-*O*-glucosyltransferase (UFGT) to the candidate genes putatively involved in the vacuolar anthocyanin transport. Additionally, data on northern blot analysis revealed that other anthocyanin biosynthesis pathway genes in addition to UFGT were also expressed coordinately at higher levels in red skin grapes than in white skin grapes. Hence, molecular targets of VvMybAs may be a set of anthocyanin biosynthesis pathway genes. Therefore, we compared GeneChip data of the berry skin of 'Pinot Noir' and its white sport 'Pinot Blanc' to reveal the targets of VvMybAs. A 5-fold change cut off (p value ≤0.05) retained 126 probe sets from 12,340 probe sets flagged "present" at all of the biological replicates of at least one cultivar. Out of the 126 probe sets, 70 probe sets were up regulated, whereas 56 probe sets were down regulated in 'Pinot Noir' compared to 'Pinot Blanc'. The highly hybridized probe sets (≥5 fold) in 'Pinot Noir' were mostly related to anthocyanin biosynthesis, whereas those of 'Pinot Blanc' were related to disease resistance or of unidentified function. In addition to flavonoid-3', 5'-hydroxylase and cytochrome b5 DIF-F, the genes specific to and putatively involved in anthocyanin biosynthesis, such as UFGT, glutathione *S*-transferase, *O*-methyltransferase, and acyltransferase (serine carboxypeptidase like protein), were highly expressed in 'Pinot Noir', while their expression was negligible in 'Pinot Blanc'. This result clearly demonstrates that the expression of these genes is induced by VvMybAs. On the other hand, those involved in the common pathway of flavonoid biosynthesis, such as phenylalanine ammonia-lyase, chalcone synthase 3, flavanone-3-hydroxylase, dihydroflavonol 4-reductase, and leucoanthocyanidin dioxygenase, were also expressed in 'Pinot Blanc' to a certain extent, and their expression was enhanced in 'Pinot Noir'. This result indicates a possibility that the expression of these genes are induced by both VvMybAs and other transcription factor(s).

O-24

Metagenomic sequencing as a tool to elucidate etiology - the case of the virome of a vineyard

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Next generation high throughput sequencing is fast gaining credibility and popularity as a tool to determine the genetic composition of environmental samples, mainly because of the all inclusive and unbiased nature of analyzing completely unknown samples. Applications vary from studying microbe populations of the oceans to diagnoses of bacterial infections in humans and even elucidating the etiological complexity of virus diseases of grapevine, as described in this study. Double stranded RNA was isolated from a sample consisting of 44 pooled, randomly selected vines from a leafroll-diseased vineyard in South Africa, and used in a deep sequencing analysis to build a census of the viral population. The dsRNA was sequenced in an unbiased manner using the sequencing-by-synthesis technology offered by the Illumina Genome Analyzer II, and yielded 837 megabases of metagenomic sequence data. Multiple *de novo* assemblies were performed with the assembler Velvet using different parameter settings. The scaffolds generated by all these *de novo* assemblies were then combined into contigs using CAP3 contig assembler. Contigs and scaffolds, from some of the *de novo* assemblies, were subjected to BLAST searches against the NCBI databases and the top hit scores used for virus identification. Based on the BLAST results, full-length genome sequences were selected from the NCBI database and used as reference sequences in MAQ re-assembly analysis. Four known grapevine viral pathogens were identified; as expected *Grapevine leafroll-associated virus* 3 was found to be the most prevalent, followed by *Grapevine rupestris stem pitting-associated virus* and *Grapevine virus A*. *Grapevine virus E*, a virus not previously reported in South African vineyards, was also identified in the study. Moreover, viruses not previously identified in grapevine were detected. The second most prevalent virus detected in the environmental sample was a member of the Chrysoviridae family, similar to Penicillium chrysogenum virus. Sequences aligning to two other mycoviruses were also detected. The identification of all the viruses present in the leafroll-diseased vineyard will assist in the elucidation of the viral disease etiology. This methodology is not restricted to viral diseases and can be applied to investigate the etiology of diseases caused by other pathogens.

O-25

A gene expression map of *Vitis vinifera* 'Corvina' development

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Transcriptional programs are important in the development of multicellular organisms. In contrast to most animals, plants develop continuously, with new organs being initiated and elaborated throughout the life cycle of the organism. As a consequence, individuals consist of repeated units, which are present in many developmental stages at any given time of the life cycle. It follows that many transcriptional programs underlying the development of different organ systems are continuously active. We analyzed global gene expression during development of the plant *Vitis vinifera* 'Corvina' in samples covering many stages and different organs. Sampling entailed the collection of 18 organs, split in different stages of development. The bud was collected in four stages, from dormancy to bud burst. The prompt bud splits into three lateral buds to form leaves together with either a tendril or inflorescence. These organs were collected in three stages of development for leaves and tendrils, and four for inflorescences. The flowers were split into anthers, pollen, ovaries, petals and sepals. Berries were sampled during five stages of development. After harvest, when berries undergo withering, sampling was carried out after the first, second and third months of this withering phase. Of the differently developed berries, multiple tissues were extracted (skin, flesh and seed) to further investigate the features of this organ. The rachis was also collected in the same five stages of berry development. Stems were picked in two phases, the green and woody stages of the cane. Furthermore, tissues were collected in order to consider the *in vitro* condition of the root culture and seedling phase of the 'Corvina' cultivar. Including all biological replicates, this project entailed the hybridization of about 168 samples. We used NimbleGen 12x135K arrays, which contain 12 x 135,000 probe sets and enable us to hybridize up to 12 independent samples on a single slide. The aim of the project is to observe the expression levels of transcriptional factor genes and signal transduction components, comparing them with those of metabolic genes. Moreover, it will explain if specialized expression patterns could be caused by preferential use of entire gene families in specific developmental processes or tissue-specific responses to the environment.

O-26

Regulation of bud fruitfulness by floral meristem identity genes: unique version of the Arabidopsis model?

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Grapevine bud fruitfulness is determined by the differentiation of uncommitted meristems into either tendril or inflorescence. To study this issue, we first profiled expression in fruitful and non-fruitful buds along the same canes of a cane-pruned variety. We then carried out post-genomic analysis of master regulators of floral meristem identity (FMI) genes during development of these fruitful and non-fruitful buds. The interpretation of the results led us to analyze the effect of cytokinins on expression of these genes in tendrils, and compare their profiles in developing tendrils and during inflorescence development in bursting buds. Based on the integration of our findings, a new model will be proposed that suggests a high degree of conservation with the *Arabidopsis* model for regulation of floral meristem differentiation.

O-27

Transcriptional analysis of inflorescence and tendril development

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Tendrils and inflorescences are organs with very different biological function (climbing or reproduction) but that share a common ontogenetic origin in the Vitaceae. In fact, they can substitute for each other depending on hormonal treatments or environmental conditions, and it is common to observe intermediate structures in the vineyards. Reproductive development is strongly regulated at the transcriptional level in plants. In order to characterize the regulation of inflorescence and tendril development we have performed a transcriptional analysis along the development of these two organs, using the Grapegen Genechip™. This Affymetrix GeneChip™ contains 23000 probe sets representing approximately 50% of the annotated genes in the grapevine genome. In this experiment we analyzed inflorescences at six different developmental stages, from inflorescences dissected from stage B1 latent buds, without flower meristems, to pre-anthesis inflorescences (bearing fully developed flowers). In addition, tendrils were analyzed at three time points, from the first emerged tendril in the apex to what was considered as a mature tendril. The results of principal components analysis of the inflorescence and tendril time-course expression data showed that the first four principal components explain 82% of the total variation. Tendril expression variation was basically modelled by PC1 (41% of total variation), while more principal components were needed to explain inflorescence expression variation due to the complexity of the structures. The analysis of the inflorescence series allowed us to observe a main inflection point at the pre-anthesis stage, while the two first stages and the two intermediate stages seemed to be more homogeneous respectively. This inflection point is mainly due to the expression of a group of genes involved in chromatin packaging and remodelling, cell cycle control, and gibberellin metabolism and response. Functional analysis of differentially expressed genes between tendrils and inflorescences revealed that transcription factors are the most over-represented functional group among inflorescence specific genes, while tendril specific genes mainly encode auxin related transcription factors, cytokinin metabolism related genes and proteins with function in photosynthesis.

O-28

Haplotype structure and cluster phenotype associations for the *VvTFL1A* gene

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VvTFL1A overexpression due to transposon insertion in its promoter region causes delayed flowering and production of a ramose cluster in the 'Carignan' RRM somatic variant (Fernandez et al. 2010). In order to analyze the possible contribution of this gene to cluster phenotypic variation in cultivated grapevine (*Vitis vinifera* L. *subsp. sativa*), we have characterized its nucleotide diversity and performed association analyses among detected sequence polymorphisms and *VvTFL1A* expression and cluster traits. We sequenced a total of 3489 bp of the *VvTFL1A* gene (corresponding to 2177 bp of promoter and 1312 bp of coding sequence) in a core collection designed to maximize phenotypic variation at 50 agromically relevant traits (Barnaud et al. 2006). Nucleotide polymorphisms were highly abundant in the promoter and intron sequences of the gene and some of them revealed significant associations (P <0.01) with flowering time, cluster width and density, berry weight and *VvTFL1A* expression. Using a multivariate regression method, we showed that two adjacent SNPs at the promoter region account for 56% of gene expression variation. Three other polymorphisms corresponding to INDELs in promoter and intron regions showed major effects on flowering time, berry weight, cluster density and cluster width. The results suggest that part of the trait variation associated to those polymorphisms could be functionally explained through variation in gene regulation or structural changes in the VvTFL1A protein. Since no trace of the transposon was detected in the *VvTFL1A* promoter in any of *Vitis vinifera* accessions of the core collection, the extreme phenotypic variation observed for flowering time and cluster size in the RRM somatic variant must correspond to a unique random insertion event.

O-29

Towards understanding the mechanisms of host resistance to downy mildew disease of grapevine by using genome wide expression profiling analysis

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Downy mildew (DM), caused by pathogen *Plasmopara viticola* (PV), is the single most damaging disease of grapes (*Vitis* L.) worldwide. However, the mechanisms of DM resistance in grapes are poorly understood. To identify genes and pathways that are involved in resistance to grape DM disease Solexa, a next generation sequencing technology, was used for estimating gene expression level by deep sequencing transcripts derived from PV infected leaves of *Vitis amurensis* Rupr. 'Zuoshan-1', a DM resistant grape cultivar. Twenty-one bp cDNA tags from a near complete set of transcripts derived from control (CON) and PV infected (INF) libraries (one day after inoculation – INF1, and five to eight days after inoculation - INF2) were sequenced. Every library was sequenced in a depth of about 8 million tags. After blasting to the "annotation genes" generated by grape whole genome sequencing, a total of 14,067 genes were obtained from the CON library, 13,176 and 14,644 for INF1 and INF2 libraries, respectively. Comparative analysis between the CON and INF libraries revealed a large number of pathogen-induced up- (about 0.9% of the sequenced tags) and down-regulated (about 0.6% of the sequenced tags) genes, while 98.5% of the tags were found no prominent difference between the CON and INF samples. A good number of genes were "highly" differentially expressed between the CON and INF libraries. For example, 537 and 465 genes were found up- and downregulated at least 50-fold when comparing expression level of INF1 vs CON, while the number was 277 (up) and 107 (down) in INF2 vs. CON. In a comparison of INF2 vs. INF1, 124 up- and 294 down- regulated genes were identified. Real Time PCR was used to validate these differentially expressed transcripts and the results are generally in agreement with the Solexa-sequencing-based gene expression profiles. Pathway enrichment analysis was used to identify significantly enriched pathways for the differentially-expressed genes. Results from this study indicated that the tag-based mRNA profiling technology is a powerful tool to identify transcripts regulated by pathogen PV infection, which will enable us to better understand the mechanisms of host resistance to downy mildew disease.

O-30

Function of *Vitis* CBF transcription factors in stress tolerance

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The CBF pathway functions in the acquisition of freezing tolerance in plants. Eight *CBF* genes are present in the wild grape *Vitis riparia* (Vr). It is likely that these genes have different functions because they are present on different chromosomes and place on different clades in a dendogram produced with all CBF protein sequences known to date. This hypothesis is supported by our qualitative and quantitative analysis of Arabidopsis lines overexpressing VrCBF1 or VrCBF4. Whole plant, electrolyte leakage and chlorophyll imaging experiments showed that while both CBF1 and CBF4 transgenic lines had increased freezing and drought tolerance, CBF4 affected freezing tolerance and CBF1 affected drought tolerance to a larger degree. Microscopy revealed that this was correlated with features that could explain the increase in stress tolerance, such as smaller and thicker leaves with larger palisade mesophyll cells, a higher number spongy mesophyll cells with fewer airspaces, and a higher number of trichomes. Further phenotypic changes with a less obvious connection to stress tolerance included dwarfing, and an extension of the vegetative phase prior to flowering. Differential activation of several genes in VrCBF1- versus VrCBF4-expressing Arabidopsis plants suggests that this is because these transcription factors have different regulons. This presentation will discuss how these findings affect strategies to breed or genetically engineer wine grapes with increased freezing tolerance but without any undesirable traits.

O-31

Mapping genetic loci for tolerance to iron induced chlorosis in grapevine (*Vitis vinifera* L.)

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Iron is essential to plants for chlorophyll formation as well as for the functioning of various iron-containing enzymes. Iron deficiency chlorosis is a wide-spread disorder of plants, in particular of those on calcareous soils. In the soil matrix, Fe exists in one of two forms, Fe2+or Fe3+. However, many environmental conditions, including the high pH of calcareous soils, can result in little Fe2+ availability ("lime-induced chlorosis"). To survive in iron limiting environments, plants have evolved two iron uptake strategies, Strategy I and II. Dicot species, including grapevine, utilize the Strategy I mechanism to take up the Fe2+ ion. Strategy I plants utilize an ATPase to secrete protons from the roots to acidify the rhizosphere which aids the release of Fe from chelating agents in the soil. A root membrane reductase reduces the prevalent Fe3+ion to the biologically usable Fe2+ ion, which can then be transported into the roots of the plant where it is available for use in various cellular processes. For Strategy I plants, the iron reduction by plant roots has been identified as the rate-limiting step in iron deficiency. Strategy II plants, such as monocot species, release phytosiderophores from the roots that chelate Fe3+ ions. The entire phytosiderophore iron complex is then transported into the root system of the plant. Complex genetic and environmental interactions have made iron induced chlorosis an extremely difficult trait to study in field trials and today, the mechanisms of tolerance remain poorly understood. The aim of the experiment was to characterize the genetic basis of grapevine chlorosis tolerance under lime stress conditions. A segregating population of 138 F1 genotypes issued from an inter-specific cross between *V. vinifera* Cabernet Sauvignon (tolerant) × V. riparia Gloire de Montpellier (sensitive) was developed and grown both as cuttings and as rootstock grafted plants with Cabernet Sauvignon scions in pots of non-chlorosing and chlorosing soils. Tolerance was evaluated by chlorosis score, chlorophyll contents and growth parameters of shoots and roots. Experiments were done in 2001, 2003 and 2006 and the material from the 2006 assay was reused in 2007. The most significant findings of the trial were: (a) the soil properties strongly affected both shoot and root development; (b) there are differences in tolerance among segregating genotypes when grown as cuttings or as rootstocks on calcareous soil; (c) calcareous conditions induced chlorosis and revealed QTLs implicated in polygenic control of tolerance; (d) rootstock strongly contributes to lime-induced chlorosis tolerance and; (e) a QTL with strong effect (from

10 to 25% of the chlorotic symptoms variance) mapped on chromosome 13was identified. This QTL colocalized with a QTL for chlorophyll content (R^2=22%) and a major QTL for vigor that explains about 50% of both aerial and root system biomass variation. These findings were supported by stable results among each year of trial. These results open new insights into the genetic control of chlorosis tolerance and could aid the development of Fe-chlorosis tolerant rootstocks.

O-32

Integrating ecophysiology and quantitative genetics to analyse the control of transpiration by the rootstock under drought conditions

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Water is the main limiting factor for yield in viticulture. Vine water status also strongly impacts grape quality. The objective of this work is to analyze the genetic determinism of drought responses induced by the rootstock. The mapping pedigree used consisted of 138 F1 individuals derived from the interspecific cross of *V. vinifera* 'Cabernet Sauvignon' (CS) × *V. riparia* 'Gloire de Montpellier' (RGM). 'Cabernet Sauvignon' was the scion grafted on each rootstock of this population. The experiment was carried out in pots, in a greenhouse. Water retention properties of the substrate were primarily determined. Transpiration was evaluated daily by weighing each pot individually with a 150 scale platform. Irrigation was applied in the mid morning in order to compensate exactly for the difference between the daily water loss due to transpiration in a particular pot and the loss of water calculated to obtain the desired level of water stress. Leaf area measurements were performed weekly in order to calculate the daily transpiration per unit of leaf area. After ten days without any stress, progressive water limitation was applied for ten days and followed by a stable water deficit stress for 15 days. These phenotypic measurements were recorded in 2007, 2008, and 2009. The fraction of transpirable soil water (FTSW) was used to define the intensity of the water stress. The response curves of transpiration to FTSW in each pot were assessed and mathematically adjusted. A large variability was observed in the studied population. QTL analysis was then performed with the coefficients of transpiration response curves. The inflexion point of transpiration response curve to FTSW was used as a plasticity trait of transpiration regulation. MultiQTL software permitted us to consider statistically the three years of investigation. Stable QTLs were detected on four linkage groups. These results demonstrate that transpiration regulation of the scion by the rootstock is determined genetically. This is the first genetic quantitative study taking into account transpiration plasticity to evaluate the water stress tolerance. These results could improve the understanding of the interaction relation between the scion and the rootstock.

O-34

Cloning and characterization of the dagger nematode resistance gene *XiR1*

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The dagger nematode, *Xiphinema index*, feeds aggressively on grape roots and in the process vectors grapevine fanleaf virus (GFLV) leading to the severe viral disease known as fanleaf degeneration. Disease symptoms consist of disrupted fruit set caused by GFLV and depressed plant growth caused by root damage from *X. index*. The use of fumigants to control *X. index* in vineyards is no longer recommended because of their high cost, detrimental environmental effects and lack of effective soil penetration to control nematodes on a deep perennial root system. Therefore, resistance to *X. index* has been an important objective in grape rootstock breeding programs. We previously demonstrated that resistance to *X. index* derived from a *Vitis arizonica/girdiana* hybrid b42-26 was largely controlled by a major quantitative trait locus, *XiR1* (*X. index Resistance 1*) located on chromosome 19. Genetic studies leading to the isolation and characterization of the genes conferring resistance to *X. index* would further our understanding of resistance and assist molecular and classical breeding efforts to control *X. index*. In this report, we present the development of high resolution genetic and physical maps in the *XiR1* region as well as the isolation of the *XiR1* locus by a positional cloning approach. This study has identified the first locus responsible for ectoparasitic nematode resistance. The markers developed from this study are being used to expedite the breeding of resistant grape rootstocks.

O-35

QTL analysis of predatory mite abundance and leaf morphology traits in a hybrid grape population

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Non-glandular leaf trichomes and domatia in grapes have been shown to positively influence the abundance of several species of predatory phytoseiid mites that feed on spider mite pests. These leaf structures, therefore, indirectly contribute to plant defense. Predatory mite abundance and several leaf morphological traits were measured in a grapevine population segregating for these morphological traits and regions of the genome underlying these traits was determined through QTL analysis. A genetic linkage map was constructed from 171 progeny of the cross of two interspecific hybrids, Ill. 547-1 (*V. rupestris* x *V. cinerea*) and 'Horizon', using 1068 molecular markers. Phytoseiid mite abundance was determined for 4 years and leaf morphological traits were measured in three growing seasons. The morphological traits measured were: leaf width, leaf length, vein bristles, vein hairs, domatia size, blade bristles, and blade hairs. Domatia size is the diameter of tufts of trichomes in vein axils; bristle and hair measurements estimate the density of shorter (<0.25 mm) and more upright trichomes and longer and prostrate trichomes, respectively. A single, major QTL was identified on linkage group 1 for all years for mite abundance, domatia size, vein bristles, vein hairs, blade bristles, and blade hairs. The percentage of phenotypic variation explained by this single QTL varied across year and trait from approximately 10 to 30%. A separate QTL was identified on linkage group 5 for leaf width and length. These results provide support for the hypothesis that phytoseiid mite abundance is positively affected by leaf morphology. The molecular markers associated with the linkage group 1 QTL should be useful for marker-assisted selection within grapevine breeding programs to enhance abundance of predatory mites and the biological control of pest mites.

O-36

New insight into the genetics of color in grape

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Performing combined QTL and association mapping studies using the combination of synthetic lines and natural diversity represents an opportunity to develop the full picture of the genetic variation for quantitative traits and ultimately to identify the causal mutations. This approach was performed in order to decipher the genetic architecture of anthocyanin composition in berry skin, a crucial trait for both wine quality and human nutrition. Grapes may either be white or colored, ranging from the lightest pink to the darkest purple tones depending on the amount of anthocyanins accumulated in the berry skin. Based on the study of total content of anthocyanins in the skin of mature berries of both a segregating population of 191 progenies and a core collection of natural resources (141 individuals), we identified 2 QTLs for the traits accounting for 62% and 7.1% of the total variation, respectively. With the positional information provided through this QTL mapping, we identified in both cases *Myb* candidate genes and identified SNPs markers in high linkage with total content of skin anthocyanins. Using a multivariate regression method, we demonstrated that six polymorphisms accounted for 86% of the observed variation. All these polymorphisms either led to structural changes in the MYB proteins or differences in the *VvMybA*s promoters. Colour tones of skin are not only due to the overall pigment concentration but also to the presence of different types of pigments and in particular the level of methylation. A similar analysis was performed for the level of anthocyanin methylation in skin anthocyanins. Two QTLs were identified accounting for 37% and 17% of the trait variation respectively. Association mapping, transcriptomic analysis and enzymatic tests enabled the identification of two mutations in a structural gene functionally linked to the difference in methylation of anthocyanins. The use of natural diversity thus helped to reduce QTL to a set of QTNs, gave a clearer picture of how isogenes combined their effects to shape the quantitative traits, and revealed the relative influence of different *cis*-and *trans*-regulatory elements on these quantitative traits.

O-37

Differential expressed genes in berries of cv. Sangiovese (*Vitis Vinifera L.*) during ripening following cluster thinning at véraison

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Yield reduction and quality improvement are not always strictly related, cluster thinning is considered a technique which, involving an increase in source-sink ratio, could lead to improvement in grape berry sugar and anthocyanin concentration. In this research an integrated agronomical, biochemical and molecular approach aims to understand which mechanisms are involved, during ripening, in determining final berry composition in cv Sangiovese vines submitted to thinning at véraison (CT, 50% of total clusters removed at véraison). The yield reduction observed in CT vines implied a significant increase in leaf area/crop weight ratio, which caused an acceleration in berry ripening rate immediately after the treatment and higher values of soluble sugars at harvest compared to control vines (C). Anthocyanin amount was strongly affected by the treatment, as their accumulation increases in parallel with soluble sugars content, reaching higher values than C at harvest. Microarray analyses, carried out with a NimbleGen array assembled on the basis of the 12X *Vitis vinifera* genome sequence, allowed to discriminate between CT and C at the end of véraison (14% of 29550 genes analyzed were differentially expressed between CT and C at this stage). Genes involved in carbohydrate metabolism, response to stress and flavonoid accumulation were analyzed in depth. As a result, CT transcriptional profile was characterized by the increase in expression of genes involved in sugars storage which usually follows sugar utilization as energy source, and by a decrease in genes induced in response to oxidative stress, corresponding to the final ripening phase. Source/sink ratio increase, positively influenced anthocyanin structural and transcriptional regulatory genes and in particular those involved in anthocyanin transport, which were strongly up-regulated in CT compared to C. The results of this research confirm the key role of source/sink ratio in conditioning sugars metabolism and reveals that carbohydrates availability is a crucial issue in triggering expression of genes involved in anthocyanin biosynthesis in field condition too.

O-38

Co-Localization of QTLs for Seedlessness and Downy Mildew Resistance in Grapevine

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A genetic linkage map of grapevine was constructed using a pseudo-testcross strategy based on a cross between the seedless *Vitis vinifera* 'Crimson Seedless' and the complex hybrid 'Villard Blanc' (Seyve Villard 12-375), resistant to downy mildew. A total of 316 DNA markers, including 262 AFLP, 48 single sequence repeats (SSR), 2 SCARs (sequence characterized amplified region) and 4 minisatellites markers were used to generate a map for each parent. For both parents, 19 linkage groups were obtained, covering 1111 cM and 926 cM for 'Villard Blanc' and 'Crimson Seedless', respectively. The position of SSR loci in the obtained maps is consistent with the genomic sequence. Quantitative traits loci (QTLs) for seedlessness and resistance to *Plasmopara viticola* (downy mildew) were investigated. Two major effect QTLs for downy mildew resistance and seedlessness were mapped on the same region of the linkage group 18. These QTLs explain 25,0-55,7% and 54,0-62,4% of total variance, respectively. The $MIKC^{C}$-Type MADS box gene *VvAG3*; whose ortholog is involved in *Arabidopsis* carpel and ovule development, and located in the confidence interval of the seedlessness QTL detected on the LG 18, could be considered as a candidate gene to control seed development in grape. Co-localizations were found in the same region, between the position of the *Rpv3* locus, which is very rich in TIR-NBS-LRR resistant gene analogs, and the main QTL identified for downy mildew resistance. Our results demonstrate that the same region of LG 18 contains important genetic determinants for seedlessness and downy mildew resistance in grapevine. Moreover, assessing the allelic variation at these agronomically important *loci* provides a basis for the development of marker-assisted selection for seedlessness and downy mildew simultaneously.

O-39

Polymorphisms in *VvPel* associate with variation for berry texture

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The identification of the genes involved in traits of interest, and the understanding of its interaction with other genetic or environmental factors, are among the main aims of genetic studies on cultured plants today. Berry texture is a quality trait of great importance in table grapes, where 'crunchy' berries are especially appreciated. Nevertheless, no QTL or related gene has been described for this trait so far. In this work, the gene *VvPel*, which codes for a pectate lyase in grapevine, was selected as a candidate gene to carry out an association study. Pectate lyase is a cell wall degrading enzyme that catalyses the eliminative cleavage of de-esterified pectin, which is a major component of the primary cell walls of many higher plants. The gene was chosen because previous studies in other species have reported a relation between this enzyme and fruit softening. Different analyses were carried out to complement the association test: the population structure, the nucleotide and haplotype diversity, the protein structure, the existence of selection and the linkage disequilibrium (LD) were evaluated in the gene *VvPel*. Firstly, a core collection of 96 table grape accessions was built from a collection of 322 table grape accessions maintained in the Finca El Encín (IMIDRA), using a reiterated maximization procedure. This core collection contained the 98% of the total morphological and molecular diversity existing in the complete collection. Diverse texture parameters were measured in this collection by means of a texturo-meter and several morphological descriptors related with berry and bunch dimensions were studied. *VvPel* was sequenced in the core collection, and 32 SNPs and 15 haplotypes were identified. LD was low enough to provide a high resolution power, and neutrality tests pointed out to the possible existence of balanced selection over the gene in the collection. Some of the polymorphisms associated significantly with texture parameters, explaining part of the variation found for the trait. These results constitute the beginning of the dissection of the complex trait berry texture in table grapes.

O-40

Quantitative analysis of texture and fertility in table grape

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Berry texture and grapevine fertility are traits of major relevance for table grape (*Vitis vinifera* L.) breeding. In order to develop molecular markers linked to those traits for their use in MAS (Marker Assisted Selection), we have carried out quantitative genetic analysis in seedless x seedless table grape progeny, consisting of 78 individuals from a cross between cultivars 'Ruby Seedless' and 'Moscatuel'. A framework linkage map was constructed for each parent. The homologous linkage groups (LGs) were used to generate an integrated map spanning 1191 cM with 165 markers (120 SSR and 45 SNP) at an average distance between loci of 7.2 cM. Berry texture and fertility were evaluated in four growing seasons. Berry texture was determined using the TA.XT2 Texture Analyser. Fertility was quantified as number of inflorescences per shoot. QTL (Quantitative Trait Loci) analyses were carried out using a multiple QTL model (MQM) based on QTLs detected at least during two previous seasons via interval mapping. Both LG specific and genome-wide significance thresholds corresponding to α=0.20 were used as minimum values for QTL detection. QTLs for texture with LOD values higher than LG thresholds were consistently detected on LGs 9, 11 and 18, explaining between 7.5% and 24.5% of total variance. For fertility, a QTL with a LOD value higher than the genome-wide threshold was identified on LG 14, explaining up to 27.2% of total variance.

O-41

Gene silencing as strategy to induce grapevine fan leaf virus (GFLV) resistance in grapevine rootstocks

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Viral infections in grapevines cause physiological disorders that lead to foliar deformations, alterations in the berry color and finally reductions in productivity. Most viral infections in grapevine are disseminated by biological vectors and then by the vegetative propagation of infected material. More than ten viruses commonly infect the grapevine, and it is not rare to find two or three different viruses in one infected plant. There are not efficient chemical treatments against virus infections. A molecular strategy to induce virus resistance in plants is gene silencing. This strategy requires the transformation of plants with a short sequence of the pathogen in a way that a double-strand RNA structure is formed during transcription, initiating gene silencing in the host. The objective of this work is the induction of virus silencing in grapevine rootstocks plants in order to use it for grafting. It is hoped that the mobile silencing signal induces virus silencing in the scion. We have transformed rootstocks plants (110 Richter and Harmony) by co-culture of embryogenic and organogenic tissues with *Agrobacterium tumefaciens* carrying a silencing vector containing a sequence of the coat protein of grapevine fanleaf virus (GFLV). Twenty-three transgenic plants of the 110 Richter rootstock have been recovered, analyzed by PCR against the GFLV sequence, and propagated to obtain several plants of each line. The transgenic rootstocks have been grafted with GFLV infected plants that were positive for virus presence by RT-PCR analysis. Once the grafts were set, the RT-PCR analysis was repeated in the scion. After one month the detection of the virus has been abolished in the scion, in four of ten analyzed rootstocks lines and the same result was maintained after six months.

O-42

Transcriptional changes in response to heterologous expression of a pear PGIP in grapevine

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To suppress the virulence of *Xylella fastidiosa* (*X. fastidiosa*), the causative agent of Pierce's disease (PD), *Vitis vinifera* cv 'Thompson Seedless' (TS) plants were transformed via *Agrobacterium tumefaciens* with the pear polygalacturonase inhibiting protein (pPGIP). We have previously shown that decreased leaf symptoms and movement of *X. fastidiosa* was achieved in transgenic plants expressing pPGIP after infection of *X. fastidiosa,* as in earlier findings with *Botrytis cinerea*. Microarray analysis (Affymetrix) of leaf tissues of grapevines expressing pPGIP revealed 3007 genes with significantly altered expression (FDR adjusted $p<0.05$) when leaf tissues of transgenic and wild type plants were compared. Genes found in microarray experiments were functionally characterized using MapMan and Blast2GO analysis tools. Of particular interest were gene expression changes in phenylpropanoid synthesis and other secondary metabolic pathways, and in stress and pathogen response genes and cell wall components. Transcription of pPGIP and other differentially expressed transcripts has been validated using RT-qPCR with TaqMan probes for the most important affected genes. Selected transformed lines have been bench grafted with wild type TS to test the hypothesis that pPGIP movement through the graft union stimulates gene expression in the wild type grapevine scion.

O-43

Genetic characterization of the USDA grape germplasm collection: Challenges and lessons learned

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Germplasm collections around the world contain an enormous amount of potentially useful genetic variation that is only occasionally quantified and remains largely unexploited. The first step towards harnessing the benefits of these collections involves a thorough survey of genetic variation. Here we describe the use of modern DNA sequencing and genotyping technologies to provide a detailed assessment of the grape germplasm collection of the United States Department of Agriculture (USDA). We show that a custom genotyping array, the Vitis9kSNP array, is useful for quantifying relationships among cultivars within *V. vinifera* and for distinguishing between highly diverse wild *Vitis* species from around the world. In addition, with 6000 reliable genetic markers, the resulting genetic data can be used to accurately estimate pedigree relationships among cultivars; to identify regions of the genome that have experienced strong positive selection during domestication and breeding; and to identify genotype-phenotype associations. Accurately assaying genetic variation across an entire genus presents distinct challenges, especially in highly diverse plant species like the grapevine. We discuss these challenges and suggest that modern DNA sequencing technologies will enable us to overcome many if not all of them.

O-44

The Turkish grape *(Vitis vinifera* L.*)* SSR database

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Turkey has rich grapevine (*Vitis vinifera* L.) germplasm, possibly owing to the fact that Anatolia (Asian part of Turkey) is considered as a likely center of cultivated grapevine (McGovern, 2003) and wild grape diversity (Arroya-Garcia et al., 2006). Recently, in an attempt to preserve grape genetic resources, a grape germplasm repository called the "National Grapevine Germplasm Vineyard" has been established at the Institute of Viticulture in Tekirdağ, Turkey. This collection currently contains approximately 1150 grapevine accessions collected from different geographical regions over more than 30 years (Çelik 2000). Despite some ampelographic studies conducted on this collection, to date very few studies have been carried out for genetic characterization of this grape germplasm at the molecular level. A project (Project number: 105 G 078) has been started with the support of the Scientific and Technical Research Council of Turkey (TUBITAK-KAMAG) and the Turkish Ministry of Agriculture and Rural Affairs for the genetic characterization of this collection and development of "The Turkish Grape (*Vitis vinifera* L.) SSR Database". By the end of the project, genetic identification of the 1115 grape cultivars will have been performed with 21 SSR (Simple Sequence Repeats) loci. Within the scope of the study, genetic parameters (allele size, allele number, heterozygosity rate etc.) of grape cultivars have been identified on the basis of national and regional levels. The number of homonym grapes was detected as 143, while the number of synonym and identical grapes was 178. Out of a total of 1115 grapes analyzed, 854 cultivars were identified apart from the synonym, homonym and identical cases. Studies on the development of the associated database are currently ongoing. The data reported here can be directly compared with other studies in other countries that used these SSR markers on grape.

O-45

Genealogy investigation in over 2300 grapevine cultivars (*Vitis vinifera* L.)

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Since 1997, many studies based on the inheritance of nuclear microsatellite markers to verify or uncover the parentage of grapevine cultivars have been performed. The aim of the present study was to undertake an extended analysis using a large sample of *Vitis vinifera* cultivars held in the INRA "Domaine de Vassal" Grape Germplasm Repository (F-34340 Marseillan-Plage, France). We genotyped 5516 accessions with 20 nuclear SSR markers and checked their identity. The dataset of 2332 unique genotypes identified (without mutants) was analysed using FaMoz software. Parentages showing a LOD score higher than 18 were validated in relation to historical data. During the process of creating the unique genotype table, we identified or confirmed approximately170 cases of cultivars displaying a mutation, mainly for berry skin colour but also for other traits such as leaf characteristics, flower sex, and ploidy level. The parentage analysis first permitted us to identify the full parentage of approximately 710 genotypes resulting in i) confirmations of the pedigree as stated by the breeder (180) or in previous published studies (80), ii) partial or complete invalidations of published pedigree (90), or iii) original discoveries (390). Second, in approximately 1150 genotypes, only incomplete parentages could be determined due to the absence of complementary parents in the cultivar sample. Last, in 284 genotypes, no direct relationship with another cultivar in the collection was revealed. Compiling these parentage results improves our knowledge of the genetic constitution of the *V. vinifera* cultivars and the identification of the main progenitors involved in varietal assortment, evolution, and grapevine breeding.

O-46

Association genetics in relation with berry size variation in grapevine (*Vitis vinifera*)

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Berry size is an important trait both for yield (table grapes) and in relation to quality (wine grapes). The final size of an organ is dependent on cell number, cell size and/or number of carpel. Indeed it has been shown that cell number is a major determinant of fruit size variability in *Solanaceae* and *Prunus*, whereas a preliminary study on a small sample of grapevine genotypes suggested that the variability of berry size resulted from cell volume enlargement. At present only few genes involved in fruit size variation have been identified, mainly in *Solanaceae* and none in grapevine. Our aims are: (1) to check, in a large sample of grapevine genotypes, the relative importance of cell volume enlargement, number of cell and carpel in berry size variation, (2) to develop association genetics approaches with candidate genes for berry size and (3) to study the DNA polymorphism along the region carrying the *fleshless berry* locus (*flb*) in order to detect possible selection pressures in cultivated varieties. The *flb* locus was identified in a grapevine natural mutant which present berries with a size reduced by 20 times and circular seeds similar to wild grapevine berries. The ovaries and the berries of 3 sub-populations of cultivated *Vitis vinifera* genotypes (279), which represent the genetic and phenotypic variability of berry size, have been phenotyped. Preliminary results on a small sample of individual (27) suggested that the final berry size was determined at early stage of berry development. The ovarian size just before fertilization was variable and proportional to the final berry size. However the ovarian cell number or size seemed to explain the final berry size differently according to sub-populations. Several mechanisms and probably several genes would be involved in the variation of the berry size in these sub-populations. These data are currently under validation on the whole set of 279 individuals. In parallel, 44 candidate genes, orthologous to genes known to be involved in flower or fruit development and/or co-localizing with berry size QTLs (Quantitative Trait Locus) and/or expressed during grapevine berry development, have been partially or totally sequenced in 47 genotypes which reflect all the genetic diversity of

grapevine. Several thousands of SNPs (Single Nucleotide Polymorphims) have been identified. A subset of these SNPs will be used for genotyping the 3 sub-populations using the SNPlex technology, in order to detect a possible correlation between genotypic and phenotypic variation. Finally, in order to test the hypothesis that flb is one of the genes selected during grapevine domestication, 69 gene fragments along flb locus (1Mbp) were sequenced in a sample of wild and cultivated *Vitis vinifera*. Our results revealed a small zone showing more polymorphism in wild genotypes than in cultivated genotypes and a large zone of 171Kb showing either more polymorphism in cultivated genotypes than in wild genotypes. These intervals will be studied more in details using two approaches: gene expression studies and an improvement of the genotype sampling for the DNA polymorphism studies.

O-47

Intravarietal variability of Crljenak kastelanski and its relationship with Zinfandel and Primitivo selections

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Recently, it has been shown that Zinfandel, Primitivo and Crljenak kastelanski (CK) share identical SSR genotype so they can be considered the same variety. Zinfandel is one of the leading varieties in California. Primitivo has a similar role in the south of Italy, while in Croatia CK is an old, almost extinct and forgotten variety, but lately looked at with regained interest. The possibility of planting new vineyards with healthy clone material selected in California and Italy seems like an easy way of improving the domestic cultivation. On the other hand, the genetic value of the remaining vines of CK could be highly valuable since they represent an old genetic source of this variety exposed to particular selection pressure. If it exists, genetic variability among Zinfandel, Primitivo and CK selections might lead to the production of different types of their winesWith the aim of preserving and characterizing the remaining vines of CK in Croatia, cuttings from 14 individual CK vines were propagated into a germplasm collection along with Zinfandel clone 01 and Primitivo clone 03, obtained from the FPS, UC Davis, and three selections of Primitivo originating from Italy (clones UBA47A and UBA55A, University of Bari) and one CAC material from Vivai Cooperativi Rauscedo). All investigated accessions were analyzed using a set of six SSR loci in order to check the identity of the genotype, as well as IRAP, REMAP and AFLP markers to assess intravarietal variability. Viticultural performance of all accessions was evaluated in 2008 and 2009 and also the polyphenolic content in skins and seeds was analyzed. The SSR analysis confirmed the uniform genotype and monozygotic status of all accessions. Preliminary ampelographic analysis among the all accessions showed superior results of CK and Primitivo which were also more alike to each other, compared to Zinfandel. Polyphenolic content of skin and seeds of some CK selections was significantly greater than in other selections. Further research is necessary to eliminate viral infections from the selections of CK, which could partly be responsible for the differences observed. Limited analysis of genetic diversity using molecular markers did not reveal significant polymorphism or logical grouping among selections.

O-48

Discrimination of grapevine clones by methylation sensitive AFLP analysis

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The European wine market is the most competitive market worldwide. To maintain the competitiveness of the domestic wine industry, availability of appropriate grapevine clones that meet the present demands of viticulture and wine market is important. However, selection and set-up of a new clone is time-consuming and expensive. Discrimination of clones is important for germplasm maintenance, breeding and certification. For breeders, identification of their clones is a pre-requisite to claim property rights and to assure the return of invest by licence fees. Grapevine varieties are traditionally identified by morphological and morphometric descriptors. During recent years, molecular marker technology has been used successfully for genetic diversity studies as well as cultivar differentiation in grapes. However, distinction of clones within a variety is difficult. Even differences described by breeders after many years of clonal selection cannot be detected using the new molecular methods. It is assumed that the different behaviour of clones of a variety can be explained by mutations, having accumulated over the years, but it could be also due to non-mutagenetic changes in gene regulation. In the latter case, the DNA-sequences are identical but the phenotypes could be different, due to the methylation of regulatory elements or coding sequences (epigenetic changes). One of the most powerful molecular marker technologies is the Amplified Fragment Length Polymorphism (AFLP) approach. In the project presented, methylation sensitive primers are used in the AFLP to identify clones by different specific methylation patterns. The results showed that methylation-sensitive AFLP allows the differentiation of clones of the rootstock *SO4* and of the variety *Silvaner*, independent from location, physiological age and year of sampling. On the basis of these results, six primer combinations (PC) were selected for rootstocks and varieties respectively, yielding clone specific markers. With these primer combinations clone-specific epigenetic markers were also identified for clones of *Grüner Veltliner* and of *Kober 5BB*. The data obtained so far confirm that epigenetic changes are also responsible for diversity within grapevine varieties, in addition to stable mutations and transposon activity. Investigations on correlation of epigenetic markers and a particular phenotype are projected.

O-49

Molecular survey of Georgian traditional grapevine genetic resources

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The South Caucasus area is considered one of the main centers of origin and domestication for the cultivated grapevine *V. vinifera* L. *sativa*. The aim of this work is to study the genetic structure of Georgian grapevines by analyzing 135 Georgian grapevine accessions using 20 SSR molecular markers and to verify the contribution of Georgian germplasm to the European viticulture. Among the 135 selected samples 112 are representatives of the traditional wine making Georgian varieties and 23 were collected from several Georgian woodlands and, after visual inspection, were considered representatives of *Vitis vinifera sylvestris* subspecies. Molecular fingerprints (20 SSR loci) of all the 135 accessions were compared with data from the 2300 accessions conserved at the Institut National de la Recherche Agronomique (INRA) grape germplasm repository of Domaine de Vassal. Nei's genetic distance was calculated among the Vassal and Georgian samples and a dendrogram was built to verify the presence of synonyms and to have an overview of the structure of Georgian grapevine germplasm compared with the ones belonging to other countries. Results highlighted that most of Georgian viticulture is quite isolated from elsewhere. These results seem to confirm that the Georgian typical varieties didn't spread to other European countries and, on the other hand Georgian viticulture did not have gene flow from elsewhere. To verify the existence of parent-offspring relationships, the software ML-relate and Famoz were used to analyze the dataset (Domaine de Vassal and Georgian accessions). Interesting relationships were identified especially between Georgian samples and among Georgian and neighbor countries. The apparent isolation of Georgian germplasm makes this viticulture particularly interesting to be investigated, and for this reason we used Identity and Microsat software to describe the structure of these samples and to perform a study on allele frequencies and characterization. The results suggest that the Georgian viticulture is still strongly correlated to its geographic origin, allowing us to clearly distinguish groups of accessions belonging to different regions of the country. In the wild samples, the molecular fingerprint has revealed very few mistakes or inter-specific crossings in the pool of collected samples and highlighted that, as already verified for other regions, there is diversification among the wild and cultivated compartments, even if this separation doesn't seem to be, in the Georgian case, as robust as seen in other viticultural areas.

O-50

Allelic variation in the domestication gene VvmyA1 in natural grape populations (*Vitis vinifera* ssp sylvestris)

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Skin color is one of the most important qualities used as the basis for selection in breeding programs for wine and table grapes. The color is determinate by the quantity and composition of anthocyanins. Color-skinned cultivars accumulate anthocyanin in their skins, whereas white skin cultivars do not. Myb-related transcriptional factors genes such as *VvmybA1* regulate anthocyanins biosynthesis. Wild grape (*Vitis vinifera* ssp sylvestris) is the ancestor of the cultivated grapevine (*Vitis vinifera* ssp sativa), berries are black-colored. We have analyzed the quantity and composition of anthocyanins in wild grapes and we have found typical characteristics slightly different from cultivated grapevine. This report examines the allelic variants present in over 200 wild grape accessions from both ends of the Mediterranean basin (Iberian Peninsula and Anatolian Peninsula). Results provide evidence that variation in one transcriptional regulator has generated a new allelic series that has been not found in cultivated grapevine. Furthermore, the allelic series showed correlation with anthocyanins content. These findings provide information about the evolution of grapes since domestication took place and have direct implications in wine quality of this important crop.

O-51

Marker-assisted breeding: Identification of monoterpensynthases in grapevine (*Vitis vinifera* L.) and their potential as markers in breeding

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Monoterpenes are considered to be one of the major classes of volatile flavour components in many grapevine varieties and are typical for fruity and floral white wine. Variety character largely depends on kind and amount of monoterpenes. GC-MS analysis showed great differences among many cultivars and clones. They are significantly influenced by environment and ripening stage, which makes a systematic clone evaluation based on phenotype difficult. The objective of this study was to identify genes involved in metabolic pathways leading to characteristic monoterpenes. The biosyntheses and molecular genetics of monoterpenes have been widely studied in various species. The recently published genome of grapevine and sequences from several plant species available in databases enable us to identify and characterise potential terpensynthases (linalool-, geraniol-, α-terpineol-synthases) in grapevine. In this study several previously unknown putative terpensynthases of grapevine were identified and their potential for marker-assisted breeding assessed. A complete set of markers for relevant monoterpenes would enable marker-assisted clone selection without the influence of environment and grape ripening/ sampling stage thus increasing the effectiveness and efficiency of clone development and genotype conservation.

O-52

Functional characterization of terpene synthases of aromatic and non-aromatic grapevine varieties

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Grape-derived flavour compounds and some flavour precursors modified during fermentation, wine evolution and ageing, are fundamental in determining the organolectic parameters used to define wine quality as aromatic quality, persistency and complexity are wine characteristics that may influence consumer choice. The knowledge of the biosynthesis of secondary metabolites that contribute to grape and wine flavour is not well understood and only a few genes involved in berry flavour biosynthesis pathways have been discovered and characterised. The aim of this research is the functional characterization of candidate genes thought to contribute to the volatile profiles of 'aromatic' (Aleatico, Moscato bianco) and 'non-aromatic' (Sangiovese) varieties. Samples of flowers and berries, covering developmental stages from fruit set to technological ripening, of 'Aleatico', 'Moscato bianco', and 'Sangiovese' have been analysed for their aroma content and the expression of genes that are predicted to be involved in grape sesquiterpene and monoterpene biosynthesis. The aroma compounds have been extracted by SPE and SPME procedures and analysed by GS-MS, while the expression of characterised grape aroma genes and some candidate genes have been analysed by real-time-PCR. Some of the genes studied show correlations between their expression patterns and the accumulation of certain aroma compounds during berry development. These genes have been the focus of functional characterization studies and include sesquiterpene synthases (which has expression patterns that correlate with the production of farnesene in flowers buds and open flowers) and a putative monoterpene synthase that is expressed when linalool accumulates during the ripening of all three grapevine varieties studied. These genes have been cloned in *E. coli* and the enzymatic activity of recombinant proteins is discussed. The discovery and characterization of genes that encode enzymes from grapevine flavour biosynthesis pathways and knowledge of factors that influence their expression and activity during berry development would support decisions on management of genotype, environment and viticultural practices for improving grape flavour and aroma potential.

O-53

An Integrated approach to studying the positional candidate gene 1-deoxyxylulose-5-phosphate synthase (*VvDXS*) involved in Muscat flavor determination.

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Fragrance in table grapes and a persistent and complex aroma in wine are both sought after by the modern consumer. In particular, the floral flavor typical of Muscat varieties, due to high levels of monoterpenoids (geraniol, linalool and nerol), is highly distinct and has been greatly appreciated since ancient times. Muscat flavor determination in grapevine (*Vitis vinifera* L.) has up to now been studied by evaluating monoterpenoid quantity through QTL analysis. These studies have revealed co-localization of 1-deoxyxylulose-5-phosphate synthase (*VvDXS*) with the major QTL positioned on chromosome 5. The aim of the present study was to assess the connection between the positional candidate gene *VvDXS* and muscat flavor by investigating the nucleotide diversity of *VvDXS* ORF and its expression profiles in the grape berry. Association between *VvDXS* polymorphisms and muscat flavor was evaluated through a structured association analysis. A putative causal SNP responsible for a predicted non-neutral substitution was found to be significantly associated with muscat-flavored varieties. Moreover, muscat-like aromatic mutants displayed unique heterozygous non-synonymous mutations near the mutated site of Muscat genotypes. The relationship between the transcription profile of *VvDXS* alleles and monoterpenoid accumulation was investigated over an extended period of berry development. It is possible that a particular trend in expression rather than the level of expression ratio affects monoterpenoid accumulation in Moscato Bianco. *In vivo* experiments using transgenic tobacco and *in vitro* enzymatic assays suggest that the putative causal SNP raises monoterpenoid accumulation by changing the 3D protein structure and by increasing VvDXS activity in Muscat enzyme form.

O-54

The study of triploid progenies from crosses between diploid and tetraploid grapes

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Crossing of plants with different ploidys is one of the effective ways to obtain new seedless cultivars. In this paper, through testing the changes of the ovule weight and observing its anatomical structure, the period and mode of ovule abortion in crosses between diploid and tetraploid grape cultivars were studied and reciprocal crossing was also conducted between them. The results indicated that the proper sampling period is one of the most important factors in ovule culture, and unlike in seedless cultivars, the embryo abortion did not occur at the same time for most seeded cultivars crossed with different ploidy plants, but the ovule abortion was only individual behavior. The period of ovule abortion was correlated with the maturing period of the female parent. The abortive embryo of early-maturing cultivars occurred at five weeks after pollination, and the mid-maturing ones and late-maturing ones were at six and nine weeks after pollination, respectively. The reciprocal crossing results showed that this crossing form has great mating obstruction, and the situation was more serious when tetraploid cultivars were used as the female parent. Very few seeds from 2X × 4X had good germinability, while those from 4X × 2X lost their germinability and no seedlings were obtained. The highest germination rate and seedling survival rate were obtained when the ovule was excised 60 days after pollination, and it is easier to harvest hybrid progeny when using diploid as female parent.

O-55

Characteristics of promising muscadine grape (*Vitis rotundifolia* Michx.): Selections from the University of Georgia (U.S.A) breeding program

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Muscadine grapes have been cultivated commercially in the southeastern United States since the middle of the 18th century for both fresh consumption and wine and juice production. The University of Georgia operates the oldest and largest breeding program dedicated to the improvement of the muscadine grape. Current goals of the breeding program include the development of new cultivars which combine large berry size with perfect flowers, earlier and later maturing cultivars, berries with dry stem scars and edible skins, and increased cold hardiness. Details of eight new selections in comparison to standard cultivars are given in this paper. Five selections are targeted towards conventional fresh market production, two for organic production, and one for home garden and pick-your-own production. Three of the most promising selections; Ga. 1-1-48 (‘Fry’ × ‘Tara’), Ga. 5-1-38 (‘Supreme’ × ‘Tara’) and Ga. 5-1-45 (‘Supreme’ × ‘Tara’), are on track to be released as cultivars in the next three years. These three selections are suitable for commercial production because they have self-fertile flowers, large berry size, and a high percentage of dry stem scars.

P-1

Genetic characterization based on the best linear unbiased predictor (BLUP) of traits related to the cluster architecture of *Vitis vinifera* L.

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A characterization of the genotypic effect and the genotype × GA_3 treatment interaction *(g×t)* of several traits associated with cluster architecture was done using a BLUP analysis on a progeny of 144 individuals obtained from a crossing of 'Ruby Seedless' × 'Sultanina'. Cluster traits (23) such as length (rl), fresh weight (rfw), number of internodes (rni), lateral shoulder length (rsl), peduncle diameter (pd), and total number of berries (tb) were measured. BLUP values were calculated according to a model selected using Akaike and to Bayesian criteria and tested by likelihood ratio. GA_3 significantly modified the phenotype distribution curve of several traits by increasing the variances and the medians. The genotype effects were significant for all the traits. The *g×t* was significant for rl and rfw. The matrix correlation and multivariate factorial analysis of BLUPs showed highly significant correlations and a latent correlation structure among the traits. The BLUPs of rl, rfw, rni, pd and rsl were all included in the first factor and those of *g×t* in the fourth factor. A cluster analysis revealed a high diversity among genotypes. From a quantitative trait loci (QTL) analysis, it was found that the linkage group 18 (LG18) in different loci harbored four significant QTLs for rl, rni and tb. LG5 harbored two significant and coincident QTLs for rfw and rsl. Considering *g×t*, one significant QTL was mapped for rl in LG14 and other for rfw in LG17. In conclusion, the different cluster architectural traits have significant genotype effects. On the other hand, the QTLs detected indicate that rl, rfw, rni, pd, rsl and *g×t* have a clear genetic basis and due to their importance in the total variance they are good determinants of the cluster architecture.

P-2

PMEI and *SPY*: Two genes with possible roles in seed and berry development in table grape (*Vitis vinifera* L.)

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Table grape (*Vitis vinifera* L.) is the most important species exported by the Chilean fruit industry. Seedlessness and berry size are important quality parameters for fresh consumption. Although progress has been made in understanding the molecular basis of stenospermocarpy, the biochemical and molecular basis underlying this process are poorly understood. To contribute to this understanding, we characterized and analyzed the expression of two genes, *PMEI* (Pectimethylesterase Inhibitor) and *SPY* (Spindly), which are strongly related to the seedless condition. Both genes were obtained by quantitative trait locus (QTL) mapping using a reference population originated from a 'Ruby Seedless' x 'Thompson Seedless' cross, which included contrasting phenotypes, i.e. seeded and seedless segregants, with large and small berries. We cloned and characterized the expression pattern of these genes in *Vitis*. The expression profile was characterized by -real time PCR and the amplification assays were performed in seven different stages of development, in four contrasting phenotypes (three individuals each) related to seed and berry development. As berry development progressed, a change in the levels of transcript was observed for most of the analyzed genes. The significance of these changes in expression will be discussed.

P-3

Identification of genes related to stenospermocarpy in table grape (*Vitis vinifera* L.)

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The stenoespermocarpy is a common phenotype in some table grapes and is characterized by the presence of seminal traces instead of complete formed seeds. This trait is highly demanded in international markets and therefore it is in the best interest of many breeders to create new seedless varieties. Genetic mapping analyses have identified a major QTL (quantitative trait loci) responsible for over 50% of the stenoespermocarpic phenotype. This QTL is located in linkage group 18, where the molecular marker VMC7F2 co-localize with VvAGL11, a MADS-box transcription factor related to ovule development. Our aim is to identify additional candidate genes involved in the stenoespermocarpy by combining phenotypic analysis, QTL mapping, transcriptomic analysis and histological studies. QTL mapping was based on an extensive phenotypic characterization using a reference population from a 'Ruby Seedless' x 'Sultanina' crossing, during 3 growing seasons. The results were partially comparable with the QTLs identified in the same or other populations evaluated.A relevant QTL for seed and berry size was mapped on linkage group 18, where the candidate gene VvAGL11 had been previously mapped. Expression analysis of this gene through berry development during two seasons, showed an increase in its expression after fruit set in seeded segregants, in contrast to the seedless ones. We also have found two other QTLs in linkage groups 2 and 8, explaining 20% of the phenotype. These QTLs have been stable during 3 seasons and we are currently saturating them with newly developed SSRs and AFLP markers to map the relevant genes. A massive sequencing (RNA-Seq) will help to give additional support to the information obtained through positional cloning. In addition, preliminary transcriptomic analyses have identified a number of genes differentially expressed when comparing segregants exhibiting extreme phenotypes for seed size. We are further characterizing the stenospermocarpic phenotype by histological analysis, which shows a defect of the ovule outer integument in seedless segregants. We will use histochemistry to further characterize all our candidate genes.

P-4

Gene-assisted selection for table grape breeding: validation of a molecular marker for seedlessness

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Within the scope of the Chilean Table Grape Breeding Program conducted by the Chilean Institute for Agricultural Research (INIA), a major candidate gene (*VvAGL11*) for seedlessness was identified in chromosome 18. This major QTL was characterized at sequence, transcriptional and genetic level in an experimental progeny derived from the cross of 'Thompson Seedless' x 'Ruby Seedless'. The identified marker explains up to 70% of phenotypic variation and had a perfect allele-phenotype association in the experimental progeny. Also, genetic experiments revealed a partial dominant behavior of the seedless allele over its seeded counterpart. In the present work we report on the validation experiments for this intragenic marker (*VvAGL11*). Association analysis was performed with a population of 14 different progenies derived from ten common seedless parental genotypes. PCR-genotyping and capillary electrophoresis were performed in these seedlings and parental genotypes to obtain genetic profiles. Phenotyping was done by weighing the seed of 150 berries selected randomly from 3 bunches. The seedless allele was in the heterozygous state in all parental genotypes. A total of six different alleles were identified within the analyzed progenies and up to 11 different genotypes were identified. Association analysis revealed that the intragenic marker (*VvAGL11*) for seedlessness can be routinely used for assisted selection, both in parents and offspring. The dominant nature of the seedless allele over most of their seeded counterpart was also confirmed in these progenies. The developed marker has the potential to increase the efficiency and efficacy in the selection process. Furthermore, the dominant effect of the seedless allele allows for the selection of parents that can generate large seedless progenies, without the imperative need of crossing two seedless varieties and the subsequent *in vitro* embryo rescue.

P-5

Inheritance of some fruit quality characters in *Vitis vinifera* grapes

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The inheritance of *Vitis vinifera* table grape qualities, soluble sugars, organic acids, anthocyanins and volatiles were investigated on three progenies, 'Jingxiu' × 'Muscat of Alexandria', 'Jingxiu' × 'Xiangfei', and 'Jingxiu' × 'Jingzaojing' in two successive years. The means of progeny sugar content were lower than mean parent values. There was no significant difference in broad-sense heritability of sugars among three progenies, although the paternal parents had different sugar levels. It indicated that high sugar contents in parents may not be prerequisite to obtain genotypes with high sugar content. The means of progeny malic acid content were higher than mean parent values, and inheritance of malic acid was strongly additive, while the means of progeny tartaric acid content were lower than mean parent values, and inheritance of tartaric acid was non-additive. It suggested that high tartaric acid content in parents would be necessary for wine grape breeding in order to improve tartaric acid content in progenies, and low malic acid content in parents would be good for table grape breeding. The proportion of anthocyanins in the maternal parent determined the proportion of anthocyanins in the offspring. But the absolute content of the maternal parent had no significant effect on progenies. The presence or absence of anthocyanins in grape skin was controlled by oligogenes, and anthocyanin content was a quantitative character controlled by polygenes. Anthocyanin contents had high broad-sense heritability, which was stable in the three progenies. However, the mean content of anthocyanins was different among the three progenies, which suggested that the white paternal parents might have an effect on the anthocyanin content in progenies. All the parental aromatic compounds existed in their progenies, and the contents of the aromatic compounds showed a high variability in the progeny populations. However, among aromas, terpenoids were only detected in the paternal parents 'Muscat of Alexandria' and 'Xiangfei', and were not identified in another paternal parent 'Jingzaojing' and the maternal one 'Jingxiu'. The presence or absence of terpenoids in berries was controlled by oligogenes, and terpenoid content was a quantitative character controlled by polygenes. Moreover, the progeny of 'Jingxiu' × 'Xiangfei' had higher terpenoid content than those from 'Jingxiu' × 'Muscat of Alexandria', indicating that the Muscat parents should have an effect on the terpenoid content of progeny populations.

P-6

Polyphenolic potential of the rare Croatian variety 'Dobricic' (*Vitis vinifera* L.)

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Croatia is very rich in grapevine (*Vitis vinifera* L.) biodiversity and many native varieties have been grown mostly along the Dalmatian coast from ancient times. Many of the old native varieties have almost vanished and Dobricic is one of them. This variety has a great potential to be used in planting new vineyards, but its enological characteristics still have not been defined and its wine is still not available on the Croatian market. Polyphenolic compounds are of profound significance for determining technological and nutritional values as well as sensorial properties of grapes and wines. In this work, the phenolic potential of Dobricic grapes has been evaluated and the influence of its polyphenols on the colour and composition of the wines has been established. Two different vineyards with this variety were considered. Three samples from each vineyard were taken at the moment of harvest. Simulated maceration assays were carried out in wine model solutions containing 12 vol% ethanol, 5 g/L of tartaric acid and 50 mg/L of SO_2, neutralized with 1/3 M NaOH to pH 3.2 in order to investigate the composition of the fraction of polyphenols that are extracted from the grape into the wine. For each sample, separate extraction assays were performed using the grape skin and seeds. The results show that grape extract of this rare native variety contains appreciable amount of total phenols (on average 3585 mg/kg), proanthocyanidins (4251 mg/kg) and catechins (2718 mg/kg) as well as anthocyanins (on average 1341 mg/kg). It was determined that grape skin is rich mainly in high-molecular-mass proanthocyanidins, while an average of 21% of proanthocyanidins is contained in the seed. The skin contained higher concentration of low-molecular-mass proanthocyanidin than the seeds, as determined by vanillin index. Wines from this variety presented high colour intensity. The correlations between the polyphenolic potential of the grapes and the extractability of the proanthocyanidins, catechins and anthocyanins into the wine during the winemaking process showed good agreement.

P-7

Impact of human selection on the allelic diversity of genes involved into the anthocyanin pathway

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Berry skin colour, and indirectly the anthocyanin content, is probably one of the agronomical traits most easily selected by human during the history of the grapevine. Consequently the anthocyanin biosynthesis pathway has very interesting features for the study of gene network evolution. Moreover, the structural genes involved in this pathway are identified and characterized and regulatory genes (*VvMybAs*) of this pathway are also known. Our recent data suggest that the genetic determinism of the total variation of anthocyanin content, and by extension the berry skin colour, in cultivated grapevine is rather due to few mutations within the sequences of the regulatory *VvMybAs* genes than mutations in anthocyanin structural genes. We have sequenced the *VvMybAs* genes on a core-collection of cultivated grapevine in order to understand the evolution of the genes at the allelic level and to identify the order of emergence of the principal mutations linked with the anthocyanin content. Secondly, we have sequenced the anthocyanin structural genes on another sample consisting of *Vitis Vinifera* L. (cultivated and wild) and other species of *Vitis* to identify the direct and indirect impact of human selection in these genes. These studies showed that berry color-associated mutations evolved from wild black alleles to a pink allele (SNP variation in coding sequence of *VvMybA2*) and to the white one (Insertion of Gret1 in the promoter of *VvMybA1*). We have also identified a significant link between the positions of genes in the anthocyanin pathway and their level of divergence. More surprisingly, comparison between values of Tajima's D and Fay and Wu's H of the wild and cultivated samples for each gene showed a different impact of the selection pattern and different selected targets within these two samples. This analysis is an interesting example for developing this kind of study at a whole genome level.

P-8

Monoterpene levels in different grapevine (*Vitis vinifera* L.) cultivars and clones during fruit ripening

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Terpenes are widespread in the plant kingdom with different functions like pest protection and attraction of insects to pollinate flowers. In white wine monoterpenes largely determine the characteristic aroma profile of a fruity and flowery grape variety. In previous studies authors could identify grape varieties by means of twelve monoterpenes and divide them into three aromatic classes: (1) varieties with a high aromatic Muscat related aroma, (2) with a fruity Riesling-related aroma and (3) varieties with a neutral bouquet [1]. The objective of this study was to characterize and compare the monoterpene contents and seasonal variation of different grape varieties and clones. Grapes of aromatic, less and nonaromatic varieties were collected during ripening periods in 2007, 2008 (2 sampling dates, respectively) and 2009 (5 sampling dates: from July 15 to October 5) at the Geisenheim Research Center. Free and glycosidic bounded monoterpenes were extracted with a solidphase- extraction-method (SPE) using Strata-x (Phenomenex)-cartridges. Glycosides were enzymatically hydrolysed and extracts were analysed by GC/MS. In this study the focus was on the monoterpenols linalool, nerol, geraniol and α-terpineol, the furanoside and pyranoside cis/trans isomers of linalooloxide, the aldehydes neral and geranial plus two diendiols. Results show large differences between cultivars and in particular two clones of “Roter Traminer”. Highest contents were found in grapes of “Siegerrebe” and cultivars of Muscat varieties like “Morio Muskat”. Only traces of monoterpenes were found in “Dornfelder” and “Chardonnay 1 Gm”. During ripening linalool increased significantly in most cultivars, while α-terpineol levels varied only slightly with time.

P-9

A molecular marker system for the identification of the colour-locus in different tissues of grapevine (*Vitis vinifer*a L.)

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Anthocyanins are red- or purple-coloured secondary metabolites present in many tissues of many plants and are a subgroup of flavonoids. Most grapevine cultivars can be divided in red and white although the colour spectrum goes from white over red and grey to blue and black. The composition from about 20 different anthocyanins and their concentration in the berry skin determine this colour-variation in grape. Each cultivar has a unique set of anthocyanins. The anthocyanin biosynthetic pathway starts from L-phenylalanin and from the important intermediate product, dihydrokaempferol two similar pathways leading either to cyanidin or delphinidin respectively. These products are subsequent glycosylized through UDP-Glc-Flavonoid-3-O-Glucosyltransferase (UFGT). The expression of the *UFGT* gene is much lower in white grapes than in red or black but no significant differences can be found in the sequence of the gene from white to red/black cultivars. The expression of the gene *UFGT* is controlled by transcription-factors VvMYBA1 and VvMYBA3. The two paralogous genes are adjacent, building a single locus inherited with berry colour. The *VvMYBA1* gene is not expressed in white berries mainly to an insertion-mutation of Gret1 (Grape retrotransposon 1) in the promoter of *VvMYBA1*.Based on this locus we used PCR-primer, both published and self designed, to develop an “easy-to-use” marker system to determine the potential berry skin colour from wooden material. But the application of the system to different tissues: wood, roots, anthers, leaves and grape skin leads to different results. Especially in the grey variety Pinot gris, which is prone for colour chimaera, has shown great differences in various tissues and white and grey coloured sectors of berry skin. The system is now used in “In vitro-Culture” experiments for developing a test for separating chimaeras. PCR-analyses showed interesting results in red Riesling, a colour mutation of white Riesling. A secondary DNA-band slightly larger than expected was found. Also Chardonnay rosé showed similar results.

P-10

Usefulness of the SCC8 scar marker linked to seedlessness in fungus resistant table grape breeding

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Resistant table grape cultivars without compromises in quality and resistance would be very advantageous for consumers. As table grape resistance sources are very limited, hybrids with pure wine grape ancestors need to be used. As in seeded × seedless crosses the proportion of seedless individuals is low, early selection by MAS is of great importance. In this study the cross of the *Run1* and *Rpv1* containing VRH 3082-1-42 (*M. rotundifolia* × *V. vinifera* BC_4) and the 'Kishmish moldvaskii' seedless table grape has been used to investigate the usefulness of SSC8 marker for MAS. The SCC8 SCAR-CAPS marker can be used to predict stenospermocarpic seedlessness, however, the *SCC8* genotypes of the parents were unknown. 78 progenies of the VRH 3082-1-42 × 'Kishmish moldavskii' cross have been tested for their *SCC8* genotypes. 'Kishmish moldavskii' transmitted its seedless phenotype to 44 individuals, which confirms the presence of a dominant *SdI* locus responsible for seedlessness. All phenotypes with any kind of disturbance in seed development have been considered seedless. The SCC8 marker results were consistent with the progeny phenotypes, we couldn't identify any recombinants. Although the seeded parent VRH 3082-1-42 (BC4) showed unusual genotype ($SCC8^{+}/0$), the genotyping of the population could be carried out due to the optimal codominant genotype of the seedless parent. We tried to trace back the haplotypes of the resistance donor parent VRH 3082-1-42 to be able to use the SCC8 marker for MAS in other crosses of VRH 3082-1-42. The *SCC8* alleles of the parents have been sequenced. Based on the allele sequences it can be concluded that the dominant, seedlessness-linked $SCC8^{+}$ allele of the seeded VRH 3082-1-42 has to be considered as recombinant and the dominant feature of the allele is not caused by a single point mutation. To investigate the 0 allele on the other haplotype, four new primers were designed to the consensus sequences of the alleles, but the amplification of the allele remained impossible. Based on this, the presence of a null allele could be explained by larger DNA rearrangements instead of mutations in the priming sites.

P-11

Drawing links from transcriptome to metabolites: the evolution of Muscat aroma in the ripening berry

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Owing to the role that aroma plays in the perceived quality of grapes and wines, there is a tremendous interest in understanding how the accumulation of sensory compounds is regulated at the molecular level. To this purpose, we applied an integrative functional genomics approach by simultaneously monitoring the abundance of transcripts and metabolites in ripening berries of Moscato Bianco (*Vitis vinifera* L.).Gas chromatography-mass spectrometry analysis of thirty-two out of the most important 'impact' compounds (monoterpenoids, C_{13}-norisoprenoids, benzenoids and C_6-aliphatic compounds) in their free and bound forms was combined with a large-scale transcriptome analysis based on the use of the AROS 1.0 oligonucleotide microarray (14,562 probes) at five developmental stages from pre-véraison to over-ripening. Several exploratory methods were tested for the integration of transcript and metabolite profiles with the final goal to identify genes or groups of genes which are linked with the accumulation of specific metabolites or groups of metabolites. Pair-wise metabolite-to-metabolite and gene-to-metabolite correlations were calculated. Various types of clustering were performed by incorporating both differentially expressed genes and metabolites. Gene network analysis was assayed in order to identify items with similar patterns of co-regulation. Finally, modulated transcripts were checked for their potential co-localization with previously identified QTLs controlling the level of aromatic compounds. This work is expected to provide insight into the regulatory network of grapevine secondary metabolism and is aimed at identifying genes relevant to aroma determination both at the regulatory and catalytic levels.

P-12

The onset of berry ripening is characterized by hydrogen peroxide accumulation and lipids peroxidation

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The process of grape berry ripening is very complex and it involves the fine regulation of many different metabolic pathways. Recent analyses revealed that the process is significantly regulated at the transcriptional level and that véraison is a key regulatory moment. It is known that hormones such as abscissic acid, auxins, brassinosteroids and ethylene play an essential role in the coordination of the whole berry ripening process. We have recently reported a strong accumulation of hydrogen peroxide (H_2O_2) in Pinot Noir grapes around vèraison. The occurrence of an oxidative stress during fruit development, around the time of color change, has been observed in several other fruits (tomato, pear, raspberry, strawberry). We are currently engaged in understanding if the observed peak of ROS in the grapes is part of the ripening regulatory network. In this paper an update of our current view of this process will be given. Our results showed that in berry skin H_2O_2 accumulation is accompanied by lipid peroxidation and an increase in catalase activity. MALDI-TOF mass spectrometry of the lipid fraction indicated a site-specific peroxidation of the galactolipids, which are mainly present in the chloroplasts. Preliminary results suggest that a lipoxygenase, which accumulate at véraison, would be involved in the peroxidation process.

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P-13

Ripening-related Ethylene Responsive Factor characterization by *Agrobacterium*-mediated stable gene transfer in grapevine

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The onset of ripening in grape is characterized by a dramatic transcriptional change, but knowledge of the regulatory circuits and the transcription factors involved in this important process is still lacking. Although the grape berry is considered a non climacteric fruit, there is evidence of ethylene playing a role in fruit ripening, and we have recently identified four ethylene responsive factor (ERF) genes which are transcriptionally modulated at the veràison phase. *ERF1* and *ERF062* genes showed a great increase of expression at this developmental stage and remained highly expressed during ripening, whereas *ERF3b* and *ERF4* transcripts were very abundant in the green berries but showed a minimum of expression at veràison. These gene expression profiles suggest they are possibly involved in ripening regulation. In order to understand the function of the *ERF1* gene in grape, its over-expression and silencing were carried out by gene transfer technology in *Vitis vinifera* 'Brachetto'. The complete *ERF1* coding sequence was cloned in pK7WG2 vector for over-expression, whereas a 209 bp *ERF1* specific sequence was cloned in pK7GWIWG2 (II) for gene silencing. ‘Brachetto’ embryogenic callus was co-cultured for two days with *Agrobacterium tumefaciens* strain EHA105 carrying the binary vectors and then transferred to media with Timentin for *Agrobacterium* elimination and subsequently with kanamycin for the selection. Putatively transgenic embryos have been regenerated and induced to convert into plantlets, and molecular assays will be necessary to evaluate transgene insertion, copy number quantification, and expression.

P-14

Morphological characteristics of cracking susceptible Korean table grapes, 'Heukgoosul' and 'Tamnara'

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Though 'Heukgoosul' ('Golden Muscat' x 'Pione') and 'Tamnara' ('Campbell Early' x 'Himrod Seedless') are very promising new table cultivars bred in Korea, they have serious problems with berry cracking. The growth characteristics and morphology of berries was investigated to determine the factors affecting berry cracking among 'Heukgoosul', 'Tamnara', and cracking resistant 'Hongisul' and the two major cultivars, 'Campbell-Early' and 'Kyoho'. Cluster weight was 457.3 g in 'Heukgoosul', 331.2 g in 'Tamnara' 321.8 g in 'Hongisul', 341.0 g in 'Campbell-Early', and 506.1 g in 'Kyoho'. Berry weight was 11.19, 7.57, 5.01, 5.61, and 11.30 g respectively. The firmness of the pericarp, which influences berry cracking, was highest at 1.62 kg·Ø5mm^{-1} in 'Hongisul' compared with 1.39, and 1.38 kg·Ø5mm^{-1} of cracking susceptible 'Heukgoosul' and 'Tamnara' grapes. The berry splitting rate under critical turgor pressure was over 70% in 'Heukgoosul', 'Tamnara', and 'Kyoho' in order of 'Hongisul' < 'Campbell Early' < 'Kyoho' < 'Heukgoosul' < 'Tamnara'. Critical turgor pressure was highest of 13.2 atm in 'Campbell Early' and was lowest of 5.7 atm in 'Tamnara' grape. The length of the fruit stalk and cap stem, which influences the denseness of berries in a cluster, were shortest at 159.5 and 6.25 mm in cracking susceptible 'Heukguseul'. The breakdown of the stylar scar observed using SEM was seen in 'Heukgoosul', 'Tamnara', and 'Kyoho'. Those cracking susceptible three cultivars also had irregular arrangement with larger cells in the transverse sectional structure of pericarp. As a result of morphological observation, berry cracking in the 'Heukgoosul' grape was influenced by the higher berry density in a cluster with shorter length of fruit stalks and cap stems and in 'Tamnara' was associated with structural weakness of pericarp with lowest berry firmness and critical turgor pressure.

P-15

Characteristics of berry growth in tetraploid table grapes with different cracking susceptibilities in Korea

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Berry cracking is one of the most serious problems for high quality table grape cultivation using tetraploid cultivars in Korea. Berry cracking patterns and factors were investigated through growth characteristics and morphological observation of berry between two tetraploids (‘Fujiminori’ and ‘Kyoho’) and compared with diploid ‘Campbell Early’ which has different cracking susceptibility of berry. The stomata were scattered on ovary surface at full bloom stage and numbers of stomata were different: 16.5 in ‘Fujiminori’, 14.7 in ‘Kyoho’, and 9.95 in ‘Campbell Early’ and the stomata were scattered on ovary surface. T
The numbers of stomata at the veraison stage, however, changed depending on location of berry to 9.8 (stylar end trisection), 6.2 (equatorial trisection), and 3.6 (stem end trisection) in ‘Fujiminori’ and 5.3, 7.7, and 1.7 in ‘Kyoho’, and 2.7, 3.9, and 3.2 in ‘Campbell Early’. As berry growth advanced, patterns of distributed stomata changed and were different in each cultivar. The berry cracking rate at veraison of ‘Fujiminori’ and ‘Kyoho’ was remarkably high, 13.2 and 7.0%, compared with 0.6% in ‘Campbell Early’. Berry cracking-sensitive ‘Fujiminori’ and ‘Kyoho’ grapes showed rapid berry growth at stage III after veraison compared with cracking-insensitive ‘Campbell Early’. Depending on the locations of berry divided into the stylar end, equator, and stem-end, cracking rates were also different with 59.0, 34.5, and 6.5% in ‘Kyoho’ and 74.5, 12.5, and 12.0% in ‘Fujiminori’. After the veraison stage, larger cracking of berry was observed from the fruit lenticels which were degenerated from stomata and stylar scar in ‘Fujiminori’ compared with cracking resistant ‘Campbell Early’ grape.

P-16

A genetic analysis of berry quality traits in Spanish red wine grape

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The quality of wine is mainly dependent on the quality of the grape berry. Yield, berry ripening, berry size, acidity and sugar content, and phenolic maturity; expressed as total polyphenol index (TPI), color intensity (CI), tannin content, anthocyanin content (both extractable and total); are major traits associated with berry quality and aging of wine, respectively. These traits result from complex developmental processes influenced by genetic and environmental factors.A segregating population was constructed with 151 genotypes derived from a cross between two representative Spanish red wine varieties: Tempranillo and Graciano. Tempranillo is the most widely planted indigenous variety, present in 28 Spanish wine regions and is associated with good quality wine. Graciano is a red Rioja variety used in blends with Tempranillo; Graciano has a later sprouting and maturation date, lower productivity, higher acidity, smaller berry size, and higher phenolic content compared to Tempranillo. We have evaluated the population for three consecutive years. Phenotypic correlations among traits within years; and between years within traits were determined. Significant correlations were found between yield, CI, skin anthocyanins, total and extractable anthocyanins content, TPI, tannin and sugar content, and seed traits within years. Simultaneously, parental genotypes and progeny were screened for 62 SSRs loci and 6 AFLP primer combinations in a LI-COR IRD 4200 Sequencer. A genetic linkage map is being constructed for each parent using Joinmap 4.0. This will allow the identification of QTL responsible for traits of oenological interest in these representative Spanish red wine varieties.

P-17

Genetic analysis of wine grape high-quality ripening on the 'Monastrell' x 'Syrah' progeny

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The technological (sugar/acid balance) and the phenolic ripening (accumulation and extractability of grape anthocyanins and tannins) have a great relevance in the wine quality. In order to find genes involved in wine grape high-quality ripening, we are carrying out a genetic analysis in a F1 progeny segregating for these traits, consisting of 230 hybrids from a cross between the cultivars 'Monastrell' and 'Syrah'. 'Monastrell' is a variety widely grown and used in Murcia Region (Southeast of Spain), and very well adapted to the dry conditions of the Mediterranean climate. 'Syrah' is used in the same region blended with 'Monastrell'. We genotyped the 230 offspring for 138 SNP markers (Lijavetzky et al. 2007) and 104 SSR markers scattered all over the 19 linkage groups (LGs). The consensus map contained 229 markers covering 1180.79 cM, with an average distance between loci of 5.16 cM. The female and male genetic maps covered a total length of 1039.66 and 1027.20 cM, respectively. Grape quality traits and phenological parameters were evaluated during two consecutives years. QTL analyses are being carried out using a multiple QTL mapping model (MQM) focused on QTLs previously detected during the two seasons via simple interval mapping (SIM analysis). Both genome-wide and linkage-group-wide LOD thresholds corresponding to α=0.20 were used as minimum values for QTL detection. QTLs for sprouting time with LOD value higher than genome-wide threshold (significative QTLs) were consistently detected on LGs 1 and 8, explaining between 14% and 18% of total variance. Other QTLs for veraison time, fertility and berry weight were also found.

P-18

Use of molecular markers for seedlessness in grapevine breeding

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Molecular markers have been utilized for early selection of seedless grapevine cultivars in a grapevine breeding program of the Department of Horticulture, Faculty of Agriculture, Ankara University. A total of 415 F1 genotypes belonging to Alphonse Lavallee x Sultani hybrid combination were screened with SCC8 (Lahogue et al. 1998), SCF27 (Mejia and Hinrichsen 2003) and VMC7F2 (Cabezas et al) markers. The entire 415 F1 genotypes revealed amplification products of expected sizes when amplified with SCC8 and genotypes showing SCC8+/SCC8+ homozygote allele pattern from the products restricted with the Bgl II restriction enzyme were selected as candidate genotypes for seedlessness. A total of 81 genotypes were selected using SCF27, possessing 2.0 kb bands were also selected as candidates. Finally, genotypes possessing 198 bp allele when amplified with VMC7F2 were selected as candidates. As a result of the marker assisted selection studies, putative genotypes were selected for use in breeding studies for seedlessness as parental lines in future.

P-19

The aroma-forming substances of hybrid seedlings of grape

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The character of muscat aroma is highly valued in grape cultivars of all uses (fresh consumption and processing for wine and juice), and breeding for muscat aroma is of special importance. The study with this view in mind was based on hybrid seedlings obtained via crossing new-breds of the Institute "Magarach" with each other. The new-breds entering as the donors of the desired character were cvs 'Tsitronnyi Magaracha' also hinting of citron, 'Spartanets Magaracha' with a muscat, flowery and freshness complexity and 'Muscat JIM' (elite form) with a bright muscat aroma. The must extracts of the three new-breds and five hybrid seedlings with one of the former as a parent were investigated by gas chromatography using an Agilent Technology 6890 chromatograph fitted with a mass-spectrometric detector 5973. The substances were identified by comparing their mass-spectra with the available standards. The concentrations were calculated based on the ratios of the peaks of pentanol (5 mg/dm^3) to the identified peaks of volatile substances without using the correction factors. Terpenoid substances, esters and aldehydes were identified in the samples studied, with a total amount in the range of 3.09-8.31 mg/dm^3 depending on the sample. Linalool, its oxides and isomers, geraniol and limonene were the major terpene compounds. The highest (2.85 mg/dm^3) and the lowest levels of the total terpenoids (0.4 mg/dm^3) were found in the extracts of 'Muscat JIM' and the cross-combination with cv 'Tsitronnyi Magaracha' as a parent, respectively. The esters were found in small quantities. The identified compounds were esters of higher fatty acids with fruity and flowery aromas. Practically no differences were registered as to the quantitative ester composition of the samples studied. Non-saturated alcohols (hexanol isomers) responsible for the fresh-cut grass aroma were also identified, with the highest level in the must extract of cv 'Spartanets Magaracha', underlying the freshness component of its aroma, and the lowest levels in the cross-combinations with that cultivar as a parent. The chromatography also indicated the presence of substances masking the muscat aroma or blocking the taste sensors during the sensory analysis. This suggests that the small levels of aroma-forming components do not necessarily underlie the lower quality and pronounced muscat aroma in hybrid forms of interspecific origin provided the favorable ratios of the components.

P-20

Characterization of key components associated with the hormone signaling pathway that initiates berry ripening

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Grape berry ripening is a complex biological process, which is predominately controlled by cross-talk between different hormones and sugars. Abscisic acid (ABA) is thought to play the prominent role in the control of the onset of ripening (véraison stage). However, the precise role of ABA in the signal transduction is still poorly understood at the protein level. Recently, there are numerous lines of evidence suggesting that ABA is not the only actor in the initiation of berry ripening. Amongst several other candidates, jasmonate (JA) is believed to be one of them in the interplay involving ABA and sugars at véraison stage. Recent transcriptomic data indicate that key components of the JA signal transduction were up-regulated in grape berry throughout its development and under water deficit conditions. Recent advances in the ABA and JA signal transduction in plant model systems will be used to develop a comprehensive approach in grape berry i) to identify orthologous ABA and jasmonate signaling proteins occurring at véraison stage, ii) to determine correlations between the accumulation of ABA and JA, and their derivatives at the onset of berry ripening, iii) to assess the synergistic effects of JA, ABA and sugars in enhancing berry ripening throughout analytical profiles in grape cell culture.

P-21

Anthocyanin Profiling in the Berry Skins of Five *Vitis amurensis* Grapes and One Related Hybrid Cultivar

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V. amurensis is the world's most northerly grapevine species that originates in the exceptionally cold climate in northeast China, and many *V. amurensis* grapes have been widely used because of not only their strong cold tolerance and disease hardness, but also high pigment content. However, until now it remains no detailed description of the anthocyanin composition and content in *V. amurensis* grapes. In this study, anthocyanin profiles in the berry skins of five *V. amurensis* grapes and one hybrid cultivar were analyzed by high performance liquid chromatography-electronic spray ionization-tandem mass (HPLC-ESI-MS/MS). The result showed that both the *V. amurensis* grapes and the hybrid cultivar contained 3,5-diglucosides of anthocyanidins with high amount in addition to 3-monoglucosides of delphinidin, cyanidin, petunidin, peonidin and malvidin that are generally detected in *V. vinifera* grapes. Furthermore, pelargonidin-3, 5-diglucoside and B-type vitisins of some anthocyanidin-3-glucosides were also identified in trace amount and no acetylated anthocyanins were detected in these samples. This finding will be important to utilize this germplasm resource in grapevine breeding programs and to further uncover biosynthesis and regulation of anthocyanins in *V. amurensis* grapes.

P-22

Expression of early light-inducible proteins (ELIPs) during the transition to autotrophy of *Vitis vinifera* L. leaves

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ELIPs are thylakoid membrane proteins transiently induced by light and particularly expressed following photoinhibition. They belong to the chlorophyll a/b binding protein family and are structurally related to the light harvesting complex of photosystem II. Their function is not totally understood - some authors associate ELIPs to energy dissipation processes but others gives them a role on the protection of chlorophyll again degradation. ELIPs would bind the molecules of free chlorophyll acting as pigment carriers during plastid development. Since accumulation of chlorophyll is normally occurring in developing leaves and in grapevine young leaves normally develop under photoinhibitory conditions, in this work we analyzed the expression of ELIPs in grapevine leaves and its relationship with the rate of chlorophyll accumulation occurring during leaf transition to the autotrophy. Our results revealed that in grapevine leaves only one ELIP (VvELIP) was expressed, which would be encoded by a single-copy gene that maps to *Vitis vinifera* chromosome 5. It was also determined that the promoter of this gene presents a structure with cis-acting regulatory sequences induced by light and express following a circadian rhythm. This was corroborated by the oscillatory expression of the *elip* transcript in leaves kept under continuous illumination but previously acclimated to a 12 h photoperiod. VvELIP corresponds to a typical early light inducible protein with 199 amino acid, a 44 amino acid long chloroplast transit peptide, three transmembrane helices and a chlorophyll a/b-binding domain. After high light induction, VvELIP was highly expressed in heterotrophic leaves but very little in adult autotrophic leaves. A positive correlation was found between the level of VvELIP expression and the rate of chlorophyll accumulation. These results are in agreement with the chlorophyll protection role assigned to ELIPs which can be of importance during transition of leaves to autotrophy.

P-23

Changes of contents and antioxidant activities of phenolic compounds during gibberellin-induced development of seedless muscat grapevine

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Gibberellic acid is a plant growth regulator commonly used for increasing the productivity of seedless fruit. However, little is known about the effect of gibberellic acid on phenolic compounds in grapevine. In the present work, the effect of GA_3 application on contents and antioxidant activities of phenolic compounds in different tissues of Muscat grapevine (*Vitis vinifera L*. 'Muscat') was investigated. 100 mg L^{-1} GA_3 application could successfully induce seedless grape berries, enhance berry size, and accelerate the development of berries, resulting in earlier ripening of the seedless berry. Contents of total phenolics and total flavonoids, and antioxidant activities in leaf, stem and tentril obviously increased, while remarkably decreased in berry peel and pulp after GA_3 application. In addition, the grapevine leaf, stem, tendril and peel extracts were shown to contain high amounts of phenolics and significant antioxidant activities. Thus, these findings indicate that GA_3 application causes different effects on phenolic compounds and antioxidant activities, depending on the type of grapevine tissue.

P-24

The development of molecular markers for *Vitis riparia* to use in interspecific breeding for freezing tolerance

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One approach to incorporate freezing tolerance in a *V. vinifera* background is to cross vinifera grapes with the wild grape *V. riparia*. Ideally, one would obtain the genetic basis for freezing tolerance from the riparia but otherwise have minimal riparia genetics in the selected progeny. To speed up this process it would be desirable to have (1) a method that can analyze the (relative) freezing tolerance on young (<1 year old) progeny, and (2) molecular markers that can distinguish between riparia and vinifera chromosomes to select vinifera genetic material. For (1), we are testing whether a relative freezing tolerance ranking can be obtained using electrolyte leakage tests on leaf punches from plants that have been cold-acclimated, similarly as has been reported for Silver Birch by Li et al., 2002. An efficient electrolyte leakage test has been developed and shown not to give significant differences between non-acclimated riparia and vinifera plants. Similar tests with acclimated plants are now being performed. For (2) we are developing PCR primers that distinguish between different alleles of genes that are predicted to have a role in stress tolerance. We have amplified, cloned and sequenced alleles of many different genes from both *V. riparia* and *V. vinifera*, and designed PCR primers that distinguish between the different alleles of so far 6 of these genes. Analysis of 17 *V. riparia* accessions from different locations in Canada and the United States and several *V. vinifera* cultivars with these primers shows that the *V. riparia* accessions contain unique alleles. This presentation will report on our further progress towards designing molecular markers for at least 2 sites on each of the 19 grape chromosomes.

P-25

Improving sustainable water use in grape production using a Drought Biomarker Detection (DBD) tool

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New technologies for improving water use efficiency are especially important for agricultural sectors. In the Okanagan Valley of British Columbia, agriculture accounts for 70% of total water use, with wine growing being the third major consumer. Other grape and wine growing regions such as California and Australia are facing similar water use issues. We are currently developing a device with the specific goal of helping to improve water use efficiency by the grape and wine industries. Although our primary focus is vineyards in the Okanagan region, the technology we are developing is anticipated to have applications to other regions and crops worldwide.
Grape and wine growers rely largely on physiological markers such as visible wilting to diagnose water stress. Incorrect diagnoses can lead to over-watering and wastage of water resources, while delayed irrigation can result in temporary reductions in vine productivity or permanent vine damage. The DBD tool is envisioned to screen for biomarkers sensitive to changes in stem water potential but detectable before physiological symptoms are evident. Biomarkers are molecular factors (genes, proteins, or chemical compounds) that indicate physiological states at specific times under a defined set of conditions. The DBD is based on specific sandwich antibody detection adapted to a lateral immuno-chromatographic strip type of format, where a colored latex particle-antibody conjugate captures a drought specific biomarker, carries it along a cellulose membrane by capillary action until the free ends of the biomarker bind to the capture antibody and generates a colorful signal indicating a positive result, suggesting that the vine is healthy but irrigation is needed. If the test indicator does not reveal color, the diagnosis is that preventive irrigation is not required. The DBD will assist viticulturists to make better decisions concerning irrigation and is expected to be an improvement over current water stress diagnostic practices in its accuracy, cost, and ease of use. The DBD will contain as many reusable parts as possible to maximize sustainability and safe ease of use directly in the vineyard with minimal manipulation by the viticulturist.

P-26

Identification of ABA inducible genes from cold-hardy *Vitis amurensis* Rupr. Cv. Zuoshan-1 by suppression subtractive hybridization

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Vitis amurensis Rupr., distributed in the Northeast of China, is the most cold hardy grape species. It can tolerate temperatures as low as -40～-50 C when fully cold acclimated. Our study showed that as the temperature drops, the cold hardy"Zuoshan-1"grapevine accumulates ABA quicker and in higher concentration than less cold hardy *Vitis* species. To better understand the molecular mechanisms of ABA and its role in the grape cold hardiness, we constructed a suppression subtractive hybridization (SSH) cDNA library to isolate ABA inducible genes of "Zuoshan-1". In a total of 214 randomly picked clones, 167 were successfully sequenced from the ABA-induced library, from which 31 contigs were assembled. Blasting analysis revealed that14 contigs were homologous to *Vitis* or plant species while the other 17 contigs showed high homology to *Escherichia coli*, which were apparently resulted from *E.coli* contamination. Functional analysis indicated that the putative functions of these contigs were associated with photosynthesis, cell defense or detoxification, and signal transduction.

P-27

Identification and Applications of Polyploid in Muscadine Grape (*Vitis rotundifolia* Michx.) Breeding

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Grape (*Vitis* L.) is a multibillion industry in the United States. Florida grape industry is an undeveloped agricultural enterprise because of serious disease problems. The muscadine grape (*V. rotundifolia*) is a native species and is very important for the regional grape production. Unfortunately, all muscadine grape cultivars contain large seeds that fall short the consumers' expectation for table consumption. Seedlessness is one of the most desirable characters in developing new muscadine grape varieties. Attempts to introduce seedlessness from *V. vinifera* grapes into muscadine grapes have been made by regional breeders for decades and success has been rare due to genetic distance between these two genera. An alternative approach is to develop triploid seedless grapes. The potential value of tetraploids in the production of triploid grape has been recognized for many years in *V. vinifera*, and some varieties (*V. complex*) have been bred in Asia, particularly Japan and South Korea. The objectives of this research are to characterize tetraploids/aneuploids in muscadine grapes and to utilize them in triploid seedless muscadine grape breeding. Sixteen polyploids, including tetraploids (4x) and aneuploids, have been identified in muscadine grape (*Vitis rotundifolia* Michx.). Twelve of them resulted from colchicine treatments while the rest of them were identified from thousands of seedlings derived from open pollination of 'Darlene', 'Pam' and 'Supreme'. The polyploidy status was confirmed by flow cytometry and chromosome counting. The polyploids were also morphologically distinguished from diploid muscadine origins showing earlier bud breaking, weaker vegetative growth, shorter internodes, greener and thinker leaves, fewer succulent stems, and earlier fruit ripening. Further investigation revealed that the polyploids had lower pollen viability with more floating seeds. The polyploids are being used for hybridizing with diploids to develop triploid (3X) seedless muscadine grape cultivars.

P-28

In vitro selection of HYP tolerant cell lines from "Chardonnay" embryogenic suspension culture

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Breeding for cold and salt tolerance of woody plants such as grapevine by classical methods is time consuming. An alternative approach to improve these traits of grapevine is to induce and select somaclonal variation using *in vitro* culture strategy. Suspension cultures of "Chardonnay" Proembryogenic Mass (PEM) were radiated with 10, 40, 60, 100, 500 Gy of Co60 and then transferred to the selection medium (Murashige and Skoog medium plus 0.5 μM 2, 4-Dichlorophenoxyacetic acid and 10 mM hydroxyproline). The hydroxyproline resistant cell lines were obtained from each dose of Co60 treatment (10, 40, 60, 100 Gy) except the 500 Gy, but most of them became brown after subcultures for several times. However, a stable cell line derived from radiation of Co60 at 100Gy dose was obtained, which was subsequently confirmed by hydroxyproline resistant test and proline content. After 4-week exposure to different concentrations of hydroxyproline, the resistant cell line eventually grew vividly on the MS medium plus 0.5 μM 2, 4-D and 4.0 mM hydroxyproline, while the control line turned brown. The resistant PEM also showed a higher free proline content than the control one after treated with hydroxyproline. The "resistant" PEM line of "Chardonnay" are germinated, and the plantlets are being used for further evaluation for salt, drought and cold tolerance.

P-29

NCED expression and ABA concentrations vary with genotype in response to water deficit.

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Grape production is one of the most important agricultural commodities in the USA and is threatened by global warming. This is particularly relevant in a semi-arid state such as Nevada with a rapidly growing population competing for scarce water resources. Abscisic acid (ABA), a plant stress hormone, regulates water use efficiency and drought resistance in plants. 9-cis-epoxycarotenoid dioxygenase (NCED) is the rate-limiting step in ABA biosynthesis, through the cleavage of a C_{40} precursor into C_{15} and C_{25} products. NCED expression and ABA concentrations were determined over the course of the day. A controlled water deficit assay was developed. Preliminary results indicated varying levels of NCED transcript abundance in response to controlled water deficit in different genotypes. A large screen of 200 different genotypes is in progress. Our hypothesis is that the response of NCED to water deficit will be correlated with water use efficiency and drought resistance; that is, a larger response of NCED in different genotypes will be associated with greater drought resistance and water use efficiency. Experiments are in progress to test this hypothesis. A summary of these results will be presented.

P-30

In silico SNP detection for genes of the anthocyanin metabolism in *Vitis*

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Grape flavonoids, especially anthocyanins, are important contributors to color, taste, antioxidant and nutraceutic properties (such as protection against cardiovascular diseases and cancer) in fresh fruit and processed products. Breeding programs and wild *Vitis* germplasm banks contain wide genetic variation in anthocyanin biosynthesis- and metabolism-associated traits, indicating the complex genetic control of the process throughout plant development. Expressed sequence tag (EST) and genomic databases were investigated using bioinformatic tools to identify SNP markers present in structural and regulatory genes associated with anthocyanin metabolism in *Vitis*. We have identified more than 1,000 putative SNPs in nine structural and twelve regulatory genes of anthocyanin metabolism in *Vitis*. The data suggest that the identified genetic variation is sufficient to distinguish among *V. vinifera* cultivars and hybrids and wild species. Regulatory genes were shown to be more rapidly evolving than structural enzyme-coding sequences. The evolutionary rate of the sequences associated with regulatory factors also appears to be differential, with higher levels of conservation found in the MYB family of transcriptional regulators associated to anthocyanin regulation. After experimental validation, the predicted polymorphisms will provide tools for further mapping, genome structure, and functional studies.

P-31

Grape berry development proteome: protein extraction and resolution by two-dimensional gel electrophoresis

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Proteomic analyses have emerged in several areas of plant biology. Two-dimensional gel electrophoresis (2-DE) coupled with mass spectrometry is one of the most efficient and powerful methods to study complex patterns of gene expression at the protein level. Nevertheless, the major constraint of 2-D protein separation is the quality of the protein extracts to be assayed. The electrophoretic separation of proteins from plant tissue extracts is often complicated by other non-protein contaminants such as organic acids, lipids, polyphenols, pigments, terpenes, and other secondary metabolites. High quality whole cell protein extracts from woody plants, especially grapevine, is difficult to obtain due to the high levels of contaminants. The objective of this work was to develop protein extraction protocols to ensure a high-resolution two-dimensional gel electrophoresis for proteomic profiling from berries at different development stages: fruit set, 2-4 mm and 6-8 mm berries, pre- and post-véraison stages. It uses a mixed protocol of classical TCA/acetone precipitation and phenol extraction. The pre-treatment with TCA/acetone is a very effective precipitant, eliminating instantly proteolytic and other modifying enzymes. On the other hand phenol extraction optimizes extraction of cytosolic and membrane proteins. The high pH buffer (7.8) inhibits most common proteases, assures that the abundant phenolic compounds are mainly ionized, and neutralizes acids that are released by disrupted vacuoles. The efficiency of this combined protocol was monitored by two-dimensional gel electrophoresis. Protein was loaded on isoelectric strip gels in which the pH gradient was established with pH 4-7 ampholines (Multiphor II system). The second dimension was carried out on 12% SDS-polyacrylamide gels, and proteins were visualized by deep purple staining. This protocol is being used to extract protein for proteomic analysis from a ‘Ruby seedless’ x ‘Thompson seedless’ crossing which exhibited extreme and opposite phenotypes for seed and berry size in the scope of a Chilean private-public partnership, the Biofrutales Consortium.

P-32

Vitis vinifera genome annotation improvement using next-generation sequencing technologies and NCBI public data

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Grapevine (*Vitis* spp.) is the most widely cultivated and economically important fruit crop and exhibits a huge diversity, comprising more than 50 species. However, the main grape products are produced solely from *Vitis vinifera* whose origin is assumed to have been the southern area of Caucasus Mountains and Caspian Sea. In Chile, grapevine represents the main fruit crop and has made our country the principal exporter of fresh consumption products in Southern hemisphere. Moreover, grapevine constitutes *per se* a model for woody plant species. Our aim is to develop a national biotechnological platform to support new genome sequencing technologies capabilities for Chilean grape breeding programs, which will be used in germplasm selection for traits of interest as well as SNPs, splicing variants, and new gene identification. We analyzed data from 18 species of the *Vitis* genus, including commercial varieties of *Vitis vinifera* and wild species of *Vitis* genus obtained using Illumina sequencing technology from two RNA-seq experiments performed by Zenoni *et al*. (2010) and Myles *et al*. (2010), both available at NCBI. The first one reported data from 'Corvina' and the latter from 'Pinot Noir', inbred 'Pinot Noir' and 15 other cultivated and wild species. The sequence reads were aligned onto the 12X draft sequence of the 'Pinot Noir 40024' genome whereas the reported data was aligned onto 8X genome. The alignments were analyzed to detect alternative splicing events and expressed single nucleotide polymorphisms (SNPs). We aligned 116,665,608 reads, selecting hits with at most two mismatches. Over 55% of the reads showed only one alignment to the reference genome, 19% had multiple matches and 26% of them were unmatched. Our preliminary results detected 387,278 putative SNPs using a Q-score of 20, coverage over 8X, and variability over 25%. This study will allow us to improve the current *Vitis* genome annotation as well as increase our knowledge about *Vitis* evolutionary phylogenetic relationships. Our next step will be to search for new genes as well as splice variants within *Vitis* genus. Our future perspects involve the integration of our developing experiments from transcriptomics and proteomics approaches.

P-33

Analysis of expressed sequence tags (EST) from *Vitis vinifera* L. flower and fruit and electronic mapping of new ESTs

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Expressed Sequence Tags (EST) are a cost-effective molecular biology tool that eases our understanding of genes involved in plant growth and differentiation. ESTs represent a valuable and abundant resource for genome annotation, gene expression, and comparative genomics in plants. In this study, a total of 6,230 EST sequences were produced from 7,561 clones in the grape (*Vitis vinifera* cv. Summer Black) flower and fruit cDNA library. After cluster and assembly of datasets, 3,582 unigenes comprising of 1,070 contigs and 2,512 singlets were established. 925 unique ESTs with known or putative functions were gained by BlastX analysis against the NCBI non-redundant protein database (nr). The sequences were assigned to 12 putative cellular roles based on additional functional annotation as well as comparative and classification analysis. 381 new ESTs were gained after Blastn analysis, and 79 SSR loci were mined from these new ESTs by MISA. After electronic mapping, 289 new EST sequences could be mapped to all the 19 grape chromosomes. The three highest matching chromosomes (chr) were chr18, chr5 and chr14, with 11.76% (34), 8.30% (24) and 7.96% (23) new ESTs mapped on, respectively.

P-34

Genome-wide identification of grapevine specific genes

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Using the grapevine (*Vitis vinifera*) genome annotation, along with the eight sequenced genomes (*Arabidopsis*, *Oryza*, *Populus*, *Carica*, *Glycine*, *Medicago*, *Sorghum*, and *Zea*), the plant transcript assemblies from 250 plant species, ESTs and no redundant (NR) proteins datasets from GenBank, we identified 1,859 genes in grapevine that lack significant sequence similarity with all other species. This set of 1,859 genes is termed grapevine-specific genes (GSGs). These GSGs were screened against the entire grapevine ESTs from NCBI to identify expressed genes. We obtained 96 expressed GSGs that have the corresponding ESTs in GenBank. For the expressed GSGs, the *in silico* gene expression, protein motif were examined. The expressed GSGs set were divided into five groups and eight protein motifs were identified by the MEME program from the five group sequences. These genes may play roles in determining specific characteristics of grapevines.

P-35

Evidence of a Ubiquitin Fusion Degradation 1 gene family in grapevine (*Vitis vinifera*)

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The ubiquitin fusion degradation 1 protein (UFD1) is an important ubiquitin recognition component in the ubiquitin-mediated degradation pathway of animals and yeast, but information about its occurrence and role in plants is scarce. Three putative *UFD1* genes from grape were identified in this study. The data were confirmed by searching the publishing database from two independent grape genome projects with the obtained sequences. Three putative *UFD1* genes containing the evolutionarily conserved UFD1 domains at the N-terminus were found on chromosome 3, chromosome 6 and chromosome 12, suggesting the presence of *UFD1* genes in grapevine. These genes were compared with those in *Arabidopsis*, poplar, rice, wheat and maize through genome and EST databases mining. Unlike monocot species, having two *UFD1* genes, dicot species have three *UFD1* genes. Phylogeneically, the UFD1-like proteins from these six higher angiosperms fall into two distinct orthologous groups, which each includes a monocot clade and a dicot clade. One of the *UFD1* paralogs in grape, *Arabidopsis*, and poplar has been duplicated through a segmental duplication event.

P-36

Looking for genetic polymorphism through clonal variation of Pinot noir

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Clonal variation among cultivars of *Vitis vinifera* L. has been known formany years and its use achieved very significant gains for the wine industry. Nowadays, the basis of clonal phenotypic variation is barely known: hypothesis of genetic mutations or epigenetic modifications have nevertheless been considered. The objective of this study is to get a better insight of the genetic basis of clonal variation to develop a tool for clonal identification and selection. The current scientific context, access at the whole genome sequences of the individual PN40024 derived from Pinot noir and of a heterozygous clone of Pinot noir ENTAV-INRA® 115 and the development of the NGS (new generation sequencing) technologies, allows now exhaustive studies of the grapevine genome. Towards this aim, we used the 454 GX FLX Titanium technology on three official clones of Pinot noir ENTAV-INRA® 386, 583 and 777, selected for their different phenotypic traits. We firstly developed a nuclear DNA extraction method to limit the contamination from cytoplasmic DNA. We then realized one 454 run by clone and we obtained in mean 1 million reads of 350 bases by run. Our data sets from the 454 and the contigs from the whole sequence of Pinot noir ENTAV-INRA® 115 have been aligned on the reference genome, PN40024 with a compilation of different software: Mosaik-assembler, and RepeatMasker. Sixty-six percent of the reads obtained from each of the 454 runs have been aligned at a unique locus. Thanks to NGS technologies, we could compare a large portion of the genome with 3X coverage: i) in mean 8Mb between Pinot noir ENTAV-INRA® 115 and with each of the three others clones and ii) 0.5 Mb between the three clones themselves. The different sequence data obtained from these clones will be compared to identify the major molecular events which separate the clones analyzed. Preliminary results showed that SNPs are less important than insertions/deletions to be polymorphic between clones. We will validate this polymorphism and will develop method to perform identification of the clones.

P-37

SNiPlay, a web application for SNP analysis

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Sequencing and large scale genetic diversity projects have simultaneously increased in grapevine. As a consequence, the need to automatically detect polymorphisms and exploit subsequent sequence and genotyping data are growing accordingly. Here, we present SNiPlay, a web-based tool dedicated to SNP discovery and polymorphism analysis. It integrates a pipeline, freely accessible through the internet, combining existing software to compute different kinds of analysis. From allelic data, alignment, or sequencing traces given as input (notably using the Polymorfind program), it detects SNP and insertion/deletion events. Then it sends sequences and allelic data into an integrative pipeline able to achieve successive steps:

- Mapping on the *Vitis vinifera* ('Pinot') reference genome and SNP annotation (genomic position, intron/exon, synonyme/non synonyme)
- Haplotype reconstruction (using softwares Phase, Gevalt or ShapeIT)
- Haplotype network (Haplophyle)
- Linkage Disequilibrium (Haploview)
- Diversity analysis (SeqLib library)

It also includes a database to store polymorphism and genotyping data produced by projects on grapevine, notably SNPGrapeMap and DLVitis projects. Private access allows collaborators to explore data: SNP retrieval using various filters (genomic position, missing data, polymorphism type), comparison of SNPs between populations or other external information about accessions (geographical origin and phenotypic data), and export of genotype data in different formats. This database can collect and combine genotyping data coming from sequencing project as well as those obtained by Illumina chips and provides a section facilitating comparison between experiments. Finally, we plan to add a specific module able to manage and detect SNP from Next-Generation Sequencing data soon. Furthermore, SNiPlay is flexible enough to easily incorporate in the future new modules able to manage other kinds of analyses such as kinship, association studies, or population structure.
SNiPlay is available at *http://sniplay.cirad.fr*.

P-38

Characterization of a new hormone in grape and analysis of its influence on grape development processes

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Phytosulfokines (PSKs) are secreted, sulfated peptide hormones derived from larger pre-propeptide precursors. They are required for the proliferation and differentiation of plant cells in tissue culture and also promote cellular longevity. Recently it was realized that PSKs are involved in the attenuation of stress response and healing of wound-activated cells. Redundancy was confirmed between both PSK's receptors and several PSKα genes and phenotypes were observed only after parallel mutagenesis of several paralogs. Proteolytic processing of preproPSK precursors is dependent on a subtilisin-like serine protease. Four paralogs, of PSK and one PSK receptor (VvPSKR) have been isolated from *Vitis vinifera* and the genes were characterized. The expression profiles of VvPSK1 and VvPSK5 were analyzed in different organs, at different developmental and physiological stages, and in dormant buds subjected to oxidative and cold stresses. Transcripts are detectable in all tissues in basal levels and are induced during flowering and at fruit set, and later at *véraison*. Both genes are also highly expressed during embryo development and in dormant buds treated with dormancy release agents. VvPSK1 and VvPSKR were over expressed in embryonic cells and regenerated plants of *V. vinifera* and also in *Nicotiana tabacum* plants. The transgenic plants did not exhibit observable phenotypes. However, in contrast to cells grown in the dark, transgenic callus grown under long-day conditions exhibited yellow coloration. To further study the function of PSK in grape, we have developed an inducible RNAi construct to knock-down all four paralogs of the gene. This gene knock-down experiment is still underway. Our results support the model of stress-activated transcription, resembling the findings in *Oryza sativa* (rice) and *Arabidopsis*, where both transcriptional and post-translational modification of PSK documented.

P-39

Skin proteomic comparison among four grape cultivars with different anthocyanin contents

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The exocarp, in addition of being a barrier to prevent pathogen infections, plays a central role during grape ripening and is characterized by specific functions such as the biosynthesis of many secondary compounds (*e.g.* anthocyanins). In order to obtain further knowledge about the physiology of this tissue, we performed a comparison among the proteomes of the ripe berry skin of four genotypes that are known to differently accumulate anthocyanins (Riesling Italico, Pinot Gris, Pinot Noir and Croatina, ordered from the white to the darker red skin) collected in two different vintages. After extraction, proteins were separated by 2-DE, using a pH 4-7 linear electrofocusing gradient in the first dimension and 12.5% polyacrylamide homogeneous gels in the second dimension and stained with cCBB. The spots accounting for the most significant differences among genotypes were isolated through Forward Stepwise – Linear Discriminant Analysis performed on PCA scores and then characterized by means of LC-ESI-MS/MS. Through such multivariate statistical analysis it was possible to clearly distinguish the four cultivars as well as the order in which they were grouped may reflect both their relative anthocyanin content and their genetic relationship. Spot characterization allowed the identification of proteins involved in many physiological processes such as stress, defence, carbon metabolism, energy conversion and secondary metabolism. For some of them (e.g. enolase, isoflavone reductase) many isoforms, showing different behaviour in the four cultivars, were identified. It is interesting to note that the levels of many glycolytic and Krebs cycle enzymes showed a good association with the anthocyanin contents of the cultivars, suggesting that higher fluxes through these pathways could be required to sustain biosynthetic activities such as anthocyanin production. In addition, a good correlation was observed between anthocyanin accumulation and the expression of some enzymes involved in the flavonoid pathway (*i.e.* flavone-3-hydroxylase and leucoanthocyanidin dioxygenase).

P-40

Towards a characterization of the PR-10 gene family in grapevine

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We have started a project aimed at characterizing the expression profile and protein activity of pathogenesis related proteins of the PR-10 family in grapevine. According to a large scale microarray analysis carried out on a Combimatrix grapevine chip, the expression of several Tentative consensus annotated as similar to PR-10 proteins is strongly increased in resistant *Vitis riparia*, and to a lesser extent in *V. vinifera,* following infection with *Plasmopara viticola* (Polesani et al., BMC Genomics 2010). PR-10 proteins are induced by pathogen infection and abiotic stresses in a number of species; because they often show ribonucleolytic activity it has been suggested that they may be involved in antiviral defence; their antimicrobial activity has been tested with controversial results. Up to now, we have analyzed the expression level of four members of the PR-10 family, predicted in the *Vitis vinifera* genome, by Real-time RT-PCR, in response to *P. viticola* infection at 12 and 24 hours post-inoculation (hpi). All are barely detectable in healthy *V. vinifera* leaves but are induced, with similar levels, in response to infection. An homologous gene, that we called *Vr-PR10*, has been isolated from the resistant species *Vitis riparia* following infection; its coding sequence has been cloned from *V. riparia* cDNA and the corresponding protein expressed in *E. coli*. The purified protein showed ribonucleolytic activity *in vitro*. Using antibodies raised against the recombinant protein, which recognize the native protein from leaf tissue in Western blot analyses, a strong induction of the protein upon infection was shown. The protein is now being tested for antimicrobial and antiviral activity *in vitro* and *in vivo*.

P-41

Assessing the genetic variability of grape clones

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Grapevine (*Vitis vinifera* L.) is a long-living and woody plant grown worldwide. Its perennial nature and vegetative propagation over long periods favours accumulation and fixing of mutations within individual genotypes, which might exhibit altered phenotypes. Clonal selection, as a procedure of crop improvement, takes advantage of the identification of sports with agronomically and enologically important traits, which are vegetatively propagated to raise new grape clones. Given the high economical value, as reflected by related patenting and legal issues, their identification is of great relevance. While cultivar identification in grapevines is traditionally based on ampelographic descriptors and on reference microsatellite (SSR) profiles, clone discrimination is not possible with such tools. To allow true genetic identification of clones, new specific molecular markers have to be implemented. Given the availability of the Pinot Noir (clone ENTAV 115) genome sequence, it is timely to use techniques based on the polymorphism information of specific genomic sequences of interest, such as DNA transposons and retrotransposons. Transposable elements (TEs), which possess the capability to change their genomic location, are indeed potential source of mutations leading to clonal variation. An alternative way to assess clonal genetic diversity consists of the study of various polymorphisms at different layers; clonal mutations can occur either at both L1 and L2 layers or at a single layer. In this work, to fathom clonal genetic variability within six wine grape cultivars, we adopted three different methods. Firstly, we have carried out a Transposon Display analysis on 141 grape genotypes, which correspond to 47 clones (both registered and biotypes) belonging to the Pinot Noir, Pinot Gris, Pinot Blanc, Meunier, Teroldego and Gewürztraminer cultivars in 3 biological replicates (plants). We have tested them using 17 primers targeting specific regions (LTRs, LTR downstream or upstream, ORF) of 6 different TE families. Secondly, by using 6 triallelic SSR markers we have analysed four tissues (leaf, berry skin, flesh and root) of the 19 registered clones. Finally, we have genotyped the same set of samples by SNPlex^{TM} Genotyping System, testing about 500 electronic SNPs identified in coding and non-coding regions of the mentioned grape genome. Based on SNPlex results, we are currently resequencing the regions with a high level of polymorphism to confirm the presence of clonal mutations. Here we report the results of

the overall identified polymorphisms enabling molecular characterization of clones within international and local grape varieties.

P-42

Phenotypic plasticity in *Vitis vinifera*: how environment shapes wine

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Phenotypic plasticity can be defined as a re-programming of the genome in response to changes in the environment, resulting in new, different phenotypes. Plastic responses range from morphological modifications to drastic changes in physiology, life history and behavior, depending on the environment the organism faces. Phenotypic plasticity has been deeply studied in plants, both for its agronomical significance as well as its ecological and evolutionary implications. However, the mechanisms determining plastic changes are still largely unknown, especially for plants cultivated in open fields where the simultaneous challenge of different environmental signals leads to complex responses in terms of gene expression, metabolic rearrangements and epigenetic mechanisms. *Vitis vinifera* spp is one of the most plastic plants known, a single genotype being able to produce berries with different quality, thus different wine qualities, depending on the micro-environment where it is cultivated. Moreover, plastic responses in grapevine are one of the causes of excellent-to-poor wine vintages. In order to better understand dynamics of phenotypic plasticity in *Vitis vinifera*, we studied the behavior of a single genotype, the clone 48 of the cultivar 'Corvina', in different environments. We sampled berries at three developmental stages (veraison, pre-ripening and ripening), during three consecutive years (2006, 2007 and 2008). These were obtained from eleven different vineyards in Valpollicella, Soave and Bardolino, the three most important areas for local wine production in Verona. The transcriptome will be studied using the Nimblegen microarray platform, and differential gene expression will be correlated with central micro-environmental and agricultural features such as the soil type, altitude and orientation of rows, type of breeding, age of vineyards and type of rootstock. Furthermore, transcriptomic as well as metabolomic data will be analyzed in light of the chemical-physical characteristics of wines produced in the aforementioned vineyards, in an attempt to create a direct link between grapevine plasticity norms and the multitude of wine quality facets.

P-43

Proteomic characterization of downy mildew-infected grapevine plants

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We have carried out a proteomic 2D analysis of *Vitis vinifera* leaves cv. Pinot Noir artificially infected with *Plasmopara viticola* (30000 sporangia per ml), and collected at 24, 48 and 96 hours post-inoculation. Leaf tissues from 3 independent biological replicates were pooled. After testing different protocols for protein extraction, good quality 2D maps were obtained by performing protein extraction in a solubilizing/lysing solution containing 7M urea, 2M thiourea, 80mM citric acid, 1% C7Bz0 and 1x protease inhibitor cocktail. Gels (five per each sample) were scanned using a Bio-Rad VersaDoc 1000 imaging system and analyzed by PDQuest software (Bio-Rad) for spot detection, background subtraction and spot OD intensity quantification. After normalization and statistical analysis, about 100 spots differentially expressed were identified (fold-change ≥ 2, with $p \leq 0.05$). Identification by RP-HPLC-ESI-MS/MS analysis, revealed 72 unique proteins, 56 up-regulated and 16 down-regulated. Functional categories prevalently affected by infection were related to Calvin cycle and glycolysis (28%), oxidative stress response (19%), defence response (12%), photosynthesis (9%) and protein folding (6%). A peculiar pattern in the number of modulated proteins over time was observed: 53 and 47 differentially expressed proteins were detected at 24 hpi and 96 hpi, respectively, and only 14 at 48 hpi, none of which included in the “defence response” category, suggesting a temporary breakdown of the response, that will be the object of further investigations.

P-44

Transcriptomic characteristics of an Oriental cultivar of *Vitis vinifera* 'Koshu'

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Our previous studies have shown that 'Koshu', a native grape cultivar and an important white-wine grape in Japan, belongs to the Oriental cultivars of *Vitis vinifera*. 'Koshu' is well adapted to the humid summer in Japan, and shows higher disease resistance than Occidental cultivars. A hybridization experiment using biotin-labeled genomic DNA fragments to a *Vitis vinifera* GeneChip showed that the hybridization intensities of genomic DNA of 'Koshu' and those of Occidental cultivars ('Cabernet Sauvignon', 'Pinot noir', and 'Chardonnay') showed high correlation with a small number (about 40 out of 16,000 probe sets) of exceptions. This result showed that *Vitis vinifera* GeneChip is also useful for transcriptomic analysis of 'Koshu', even though major probe sets were designed from the EST data of Occidental cultivars, mainly 'Cabernet Sauvignon' and 'Chardonnay'. Transcriptomic analysis of berry skins of 'Koshu' during maturation (three weeks after veraison) showed that the top 30 and 200 highly hybridized probe sets contained 12 and 19 grape ripening induced proteins (GRIPs), respectively, out of 27 GRIPs on the GeneChip, which is a similar tendency to that of 'Chardonnay'. However, the comparison of the hybridization intensities of the berry skins of 'Koshu' and 'Chardonnay' from before the veraison stage to the harvest stage showed that the difference between 'Koshu' and 'Chardonnay' at the same stage is larger than that between the before-veraison stage and the harvest stage of the same cultivar. The berry skin of 'Koshu' highly expressed disease-resistance genes, *e.g.*, Fiber protein Fb 19, PR-4 type protein, Class IV Chitinase, in addition to flavonoid pathway genes ('Koshu' accumulates a small amount of anthocyanin in the berry skin) and unclassified proteins, compared with 'Chardonnay'. Thus, these transcriptomic characteristics of 'Koshu' are possibly one reason for the disease resistance and other characteristics of 'Koshu'.

P-45

Preliminary observations on the role of sirtuin genes in grapevine physiology

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The sirtuin/Sir2 (Silent information regulator 2) family of NAD+-dependent deacetylases and mono-ADP-ribosyltransferases play a part in various aspect of cellular metabolism in eukaryotes. Their role in mediating the calorie restriction effect extending longevity and extending lifespan in *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, and *Drosophila melanogaster* is well known. Information about their occurrence and role in plant genomes, however, is scarce. Recently, two putative sirtuin genes encoding a SIRT4-like protein on chromosome 7 and a SIRT7-like protein on chromosome 19, were identified in the *V. vinifera* genome. These two genes appear to be present in single copy as shown by Southern blotting analysis.
RT-PCR results demonstrated a baseline transcription level of both genes in different plant organs. Sirtuin discovery in the grape genome is intriguing because stressed grapevine cells synthesize compounds with hydroxylated *trans*-stilbene ring structure, such as, resveratrol (*trans* 3,3′,5-trihydroxystilbene) which is able to activate sirtuins in yeast, in *C. elegans* and *D. melanogaster*. The aim of our research is the identification of the function and activity of both putative sirtuin proteins in grapevine. We will accomplish this by investigating their interaction with resveratrol using *in vitro* and *in vivo* approaches and assaying changes in their expression levels in response to various abiotic stress conditions. *In vitro* assays demonstrated a very weak NAD+-dependent deacetylase activity of both sirtuins and no activation by resveratrol. Moreover, neither protein displayed ADP-ribosyltransferase activity. The *in vivo* function will be investigated by transforming whole grapevine plants and cell cultures and by the omplementation of a *S. cerevisiae sir2Δ* mutant.

P-46

Defense response to downy mildew in grapevine: Learning from transcriptomics and metabolomics

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Grapevine (*Vitis vinifera* L.) is the most widely cultivated and economically important fruit crop in the world. With the global warming, fungal infections are becoming higher risk factors for wine producers. *Plasmopara viticola* [(Berk. and Curt) Berl. et De Toni], the causal agent of grapevine downy mildew, is one of the most devastating pathogens in viticulture. Fungicide application during the vegetative period has become obligatory since *Vitis vinifera* varieties are generally susceptible to this disease. To better understand the mechanisms underlying disease tolerance/resistance, cDNA microarrays and H^1 NMR-based metabolomics analyses were performed. Transcriptional and metabolic changes during the defence response of *V. vinifera* `Regent´ (a resistant grapevine cultivar) were accessed and compared to susceptible cultivar 'Trincadeira'. Both cultivars were inoculated with *P. viticola* and analyzed at the following time points: 0h, 6hpi, 12hpi, 18hpi, 24hpi and 48hpi. So far, 22 transcripts were found to be differentially expressed at 0h. At 6hpi, 96 transcripts were found to be differentially expressed (54 up-regulated and 42 down-regulated). The main changes regarding the defense response at 6hpi are observed in the resistant cultivar. The transcript regulation suggests that the resistant cultivar perceives and reacts to the pathogen before the susceptible one. The multivariate data analyses, like Principal Component Analysis (PCA) and Partial Least Square-Discriminant Analysis (PLS-DA), of the ^{1}H NMR spectra showed evident innate differences between the resistant and susceptible cultivars in terms of primary and secondary metabolites possibly associated with the acquired resistance of grapevine against the pathogen. This study may contribute to the defining molecular and chemical markers of resistance to downy mildew in

grapevine which will eventually be of high value for the rapid and precise selection of putative resistant genotypes in breeding programs.

P-47

Grape berry cuticle under water deficits: morphological, metabolomic and proteomic analysis

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The cuticle membrane is an essential structure of high functional and ecological importance and is involved in the development of some disorders in skin of fruits. The cuticle restricts water loss between the epidermis and adjacent environment of the plant and protects against nutrient leaching, mechanical damage, and invasion by pathogens. Fruit cuticle composition influences berry weight loss during maturation and may determine the efficacy of alcoholic fermentative process during wine making, as it is 30% composed by the cyclic terpenoid oleanolic acid, a compound known to be important in such processes. Despite the importantce of fruit cuticles, little is known about its synthesis, assembly and metabolism. Recent reports show that cuticular wax deposition and composition influences drought tolerance. In addition, the lack of some flavonoids compounds was associated with alterations in cuticle thickness, cutin content and wax composition in tomato fruit cuticle. Combined with metabolic and morphological characterization, proteomics will provide a quantitative characterization of protein dynamics in response to water deficit and contribute to the identification of potentially biological players involved in the transport, deposition or modification of the cuticle. We studied the berry cuticle in three phenological stages (pea size, véraison and full maturation) under two water irrigation conditions (fully irrigated and non irrigated grapevines) by analyzing cuticle flavonoid composition, proteome and morphology. Berry skins from non-irrigated grapevines showed a different morphology, composition and more epicuticular waxes than those from fully irrigated ones.

P-48

‘Cioutat’: a somatic variant of ‘Chasselas’ exhibiting a programmed cell death-like phenotype

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Somatic genetic variation constitutes an important source of phenotypic variation in grapevine. In perennial plant species where mutants are difficult to generate and screen, spontaneous somatic variants represent a unique resource to understand the genetic control of complex developmental patterns. ‘Cioutat’ is somatic variant of cultivar ‘Chasselas’ displaying parsley-like leaves. Genetic analysis of progeny of a ‘Cioutat’ self-cross population showed 25% plants with wild-type (‘Chasselas’) phenotype, consistent with the hypothesis that the phenotype is caused by a single dominant mutation. Transcriptomic comparison of ‘Cioutat’ and ‘Chasselas’ revealed significant over-expression of a BAG (Bcl-2 associated athanogene) gene closely related to the *Arabidopsis AtBAG6* gene in the ‘Cioutat’ leaf primordia. We named that grapevine gene as *VvBAG6*. Interestingly, in *Arabidopsis*, *AtBAG6* is suggested to be a stress upregulated protein involved in programmed cell death (PCD). Moreover, a quantitative evaluation of the ‘Cioutat’ phenotype in the self-cross segregant population showed a positive and statistically significant correlation between *VvBAG6* expression level and the extension of the parsley leaf phenotype evaluated in the same plants. Collectively, these results suggest the involvement of PCD in the generation of the ‘Cioutat’ phenotype.

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Characterization of the grape *GAI* promoter in *Arabidopsis*

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The *GAI* (*GA insensitive*) gene plays a central role in plant GA (gibberellin) signaling. Mutations in a *GAI* gene can result in changes of plant architecture and other traits, thus providing potential opportunities for crop improvement. As a step towards exploring *GAI* variants for the improvement of grapevine (*Vitis vinifera*), we cloned a 2 kb promoter region of the grape *GAI* gene (*VvGAI*) and characterized its activities in *Arabidopsis thaliana* along with that of a 2 kb Arabidopsis *GAI* promoter (*AtGAI*) and the 35S cauliflower mosaic virus promoter (*35S*). All three promoters were fused to the *GUS* reporter gene (*ß-glucuronidase*) (designated as *pVv::GUS*, *pAt::GUS* or *p35S::GUS*). GUS expression activity of the three constructs was visually examined at both seedling and reproductive stages in transgenic *Arabidopsis* plants. Our results demonstrated that both *VvGAI* and *AtGAI* promoters showed strong activities in actively growing tissues such as young shoots and new leaves, root tips, lateral root primordial, and young inflorescences. The *GAI* promoter activity was significantly reduced in older leaves and roots and stems. This suggests that there is a very tight developmental control of *GAI* expression in plants. Some subtle differences were observed between *VvGAI* and *AtGAI* promoters in the reporter expression activities in the stamen filaments of flowers. The *pVv::GUS* construct had much stronger expression in stamen filaments than *pAt::GUS*. This observation was interesting as it might suggest that the regulatory control system for the same gene could vary in different species. A major difference in expression activities between *35S* and *GAI* promoters was found in roots. At seedling stages, strong reporter expression was observed for *35S::GUS* in all root tissues including primary, lateral and tertiary roots, but there was almost no reporter expression in the primary roots of in either *pVv::GUS* or *pAt::GUS* plants.

P-50

Estimation of protein spot variability in 2-D gels

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A common method for quantifying proteins is to separate them by two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) and quantify the intensity of the spots with image analysis software. Protein spot variability can be quite high averaging about 60 to 80% for three biological replicates. Gel to gel variability is quite high requiring the researcher to make many technical replicates and select from the best of the gels made. Our lab decided to determine where most of this variability came from. We designed an experiment to determine if the variability came from the biological replicates, protein extractions or the custom made 2-D PAGE gels. A total of 27 gels were run; there were 3 gels of 3 extractions of 3 biological replicates of Cabernet Sauvignon shoot tips. Nearly 500 common protein spots were quantified on all 27 gels. Variability was quantified with the coefficient of variation (CV). The average CV was highest for the gels and lowest for the biological replicates, averaging 55, 33 and 25% for gels, extractions and biological replicates, respectively. The CV ranged from 5 to 122% for some protein spots indicating that for many proteins it is difficult to get good quantitative data for comparative purposes. The variability of this method limits the number of proteins that can be quantified adequately and inflates the cost and labor due to the replacement of bad gels. A similar analysis was performed on buds of Seyval and *Vitis riparia*. The average CV of 861 spots was 68%. Difference Gel Electrophoresis (DIGE) is designed to reduce this variability and we tested DIGE on bud samples to determine if protein spot variability was significantly reduced. A comparison of the two methods will be discussed.

P-51

Berry skin development of the grape variety Norton exhibits distinct expression patterns of genes in defense and flavonoid pathways and unique profiles of flavonoid compounds

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The grape variety Norton (*Vitis aestivalis*) has a higher level of disease resistance than Cabernet Sauvignon (*Vitis vinifera*), and its berry chemistry is different from Cabernet Sauvignon. We conducted a microarray study on transcriptome changes of Norton berry skin from 49 days after bloom (DAB) to a fully ripened stage at 118 DAB. Samples of six berry developmental stages were collected. Microarray analysis indicated that there was a dynamic modulation of NBS-LRR resistance genes and pathogenesis-related (PR) genes, and transcript levels of the PR-1 gene clearly increased during Norton berry skin development. The majority of genes in the phenylpropanoid pathway were up-regulated; the progressive increase of transcript levels of the stilbene synthase gene family was observed during the ripening phase of Norton berry skin. Transcriptional patterns of Myb transcription factors and structural genes of the flavonoid pathway and profiles of anthocyanins and proanthocyanidins (PAs) in the berry skin development were analyzed comparatively in Norton and Cabernet Sauvignon. It was found that the total anthocyanin content in Norton berry skins are more abundant than Cabernet Sauvignon, and the kinetics of five anthocyanin derivatives and three PA compounds exhibit distinctive profiles in Norton berry skin. Transcript levels of *MybA1* reached to a peak level at 57 DAB (véraison) in Cabernet Sauvignon and at 85 DAB in Norton. *MybPA1* transcripts increased significantly at 70 and 85 DAB, and afterwards declined to baseline levels in Norton. *MybA2* and *MybPA2* transcripts reached a peak level at 57 and 71 DAB, respectively, in Cabernet Sauvignon berry skin. Transcriptional patterns of *Myb5A* and *Myb5B* were similar across the berry skin development between the two varieties. The results indicate that *MybPA1* plays a major role in Norton while *MybPA2* in Cabernet Sauvignon in the regulation of flavonoid biosynthesis. Similar pattern of *Myb5a* and *Myb5b* regulation demonstrated that general flavonoid pathways are regulated similarly between the two grapevines. The more abundant accumulation of anthocyanins and PAs in Norton than in Cabernet Sauvignon berry skin correlated well with elevated transcript levels of genes encoding flavonoid-3′-hydrooxylase, flavonoid-3′,5′-hydrooxylase, leucoanthocyanidin dioxygenase, UDP-glucose:flavonoid 3-O-

glucosyltransferase, anthocyanidin reductase, leucoanthocyanidin reductase (*LAR*) *1* and *LAR2* in Norton.

P-52

Establishment of virus-resistance in grapevine rootstocks

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Grapevine is affected by a multitude of viruses, leading to a loss of yield and quality of the grapes. The most destructive virus diseases worldwide are grapevine fanleaf and grapevine leafroll. An effective and sustainable protection against harmful viruses is to breed resistant elite and rootstock varieties. However there is no natural source of resistance available to be introduced by classic crossbreeding programmes. For this reason, RLP AgroScience is applying transgenic strategies (induction of gene silencing, expression of scFv-antibodies) to establish virus resistance both in elite and in rootstock varieties. In a project funded by the EU (FP5) and in cooperation with the Federal Association of Vine Plant Producers and a grapevine nursery, various gene constructs were developed, expressing i) the core region of the movement protein, ii) inverted repeats and iii) scFv-antibodies, in order to establish virus resistance against nepoviruses (GFLV, ArMV, RpRSV). Almost 100 transgenic lines of the rootstocks (SO4, Binova, 125AA, Richter 110) containing the different gene constructs have since been developed. The complete integration of the constructs has been confirmed in most of the transgenic lines by molecular characterisation. Between one and five copies of the transgenes were detected. In Northern analysis, transcripts of the relevant sequences were found in some but not in all transgenic lines. Detection of siRNA resulting from RNA-silencing of the virus-derived sequences is in process. Virus resistance of the transgenic lines is evaluated by laboratory screening methods, developed at AgroScience (dual culture of nematodes and candidate lines, detection of silencing by the GFP-sensor). Furthermore, transgenic lines are cultivated in a Saran-house equipped with soil containers, which were inoculated with Grapevine Fanleaf Virus (GFLV)-infected nematodes *Xiphinema index*. An overview about molecular analysis and the evaluation experiments for virus resistance of the transgenic lines will be presented.

P-53

Marker-free transgenic grapevines using the Cre/lox recombination system

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The production of transgenic plants usually requires the application of selectable marker genes, enabling the specific selection of genetically modified cells and their regeneration to intact plants. Antibiotic resistance marker genes (ARMG) generally used for selection are insignificant for the practical use of the plants. In the scope of a network project funded by the Federal Ministry of Education and Research (BMBF) on the subject “optimisation of biological safety of transgenic plants”, the Cre/lox recombination system of the bacteriophage P1 was tested by RLP AgroScience to eliminate the ARMG from grapevine. For this reason, a vector system was developed allowing the removal of the ARMG by induced activity of the Cre-recombinase after transformation and regeneration. The essential feature of the construct is the concerted removal of the co-localised ARMG and *cre*-Gene cassettes from the genome by a sequence-specific recombination event. For the easy detection of a successful recombination, the coding sequence of a grapevine myb-transcription factor was inserted as a "gene of interest". In the case of recombination, this myb-factor activates the biosynthesis of anthocyanin, resulting in red coloured cells. The proof-of-concept was verified both in the model plant tobacco and in embryogenic grapevine cell culture.

P-54

Towards a deep understanding of the function of grape flavonoid regulators VvMYB5a and VvMYB5b

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In grapevine, the biosynthesis of flavonoids is highly regulated in order that different compounds are produced in different organs and in stages of development. Despite the flavonoid biosynthetic pathway has been well studied, little is known about the transcriptional mechanism that regulates it. Recent studies suggest that two MYB transcription factors, VvMYB5a and VvMYB5b, are responsible for the regulation of the early flavonoid structural genes in different stages of berry development. Until now, the role of VvMYB5a and VvMYB5b has been mainly inferred by expression analyses and/or ectopic expression in model systems like tobacco and tomato. In petunia the mutation of the ortholog of *VvMYB5a* and *VvMYB5b (PhPH4*) results in the increase of vacuolar pH and, consequently, in blue shift of the flower color. We used functional complementation analyses of some well characterized petunia anthocyanin/pH regulatory mutants to gain information about the role of VvMYB5a and VvMYB5b in the regulatory network operating in *Vitis vinifera*. The coding sequence of VvMYB5a and VvMYB5b was fused to the constitutive promoter 35S and transformed into petunia *ph4*- mutant. Analyses of transgenic plants revealed full complementation phenotypes attributable to an activation of target genes belonging to vacuolar acidification. On the other hand, when the coding sequence of VvMYB5a and VvMYB5b was overexpressed in *an2* (acyanic) and in wild type (cyanic) petunia backgrounds, the flavonoid pathway was activated, but only in the acyanic line. Overall, specific and/or overlapping effects of the heterologous expression of the two regulators could be observed. Functional studies using heterologous systems may give results that do not reflect the real role of a gene in its homologous background. For this reason, *Vitis vinifera* cv Shiraz has been transformed to silence and overexpress both *VvMYB5a* and *VvMYB5b*. Analyses of transgenic plants (underway) will provide more conclusive information about the role of VvMYB5a and VvMYB5b in the regulation of the flavonoid biosynthetic pathway.

P-55

Agrobacterium rhizogenes-mediated induction of transgenic hairy roots in *Vitis* species

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Agrobacterium rhizogenes-mediated induction of transgenic hairy roots was previously demonstrated in *Vitis vinifera* L. and a few other *Vitis* species. In this study, 13 *Vitis* species, including *V. aestivalis*, *V. afghanistan*, *V. champinii*, *V. doaniana*, *V. flexuosa*, *V. labrusca*, *V. nesbittiana*, *V. palmata*, *V. piasezkii*, *V. thunbergii*, *V. treleasii*, *V. vinifera* L, and *V. xnovae angelae* were screened for hairy root induction by co-cultivating *in vitro* plants with virulent *Agrobacterium rhizogenes* 15834, A4, or K599 harboring binary vector pBIN61-EGFPHA, which produces green fluorescent protein signal for visual examination of transgenic hairy roots. Frequencies of callus formation and transgenic hairy root induction were recorded daily for 2 weeks after *in vitro* plants had been inoculated. The results showed that *V. labrusca* (var. 'Concord') had the highest frequency of transgenic callus and hairy root induction. Hairy roots were also successfully induced for *V. treleasii*, *V. champinii*, *V. cinerea*, and *V. vinifera*, but their frequencies were much lower than that for *V. labrusca.* In contrast, no hairy root was generated from other species during the 2-week observation period. In addition, we found that the developmental stage or age of plant material also played an important role in transgenic hairy root induction. Our results showed that hairy roots could be induced by 15834 and A4 strains with similar transformation efficiencies, however, K599 was found not to be suitable for hairy root induction in the *Vitis* species. In general, the transgenic hairy roots appeared to be bright white and much thicker than nontransgenic roots and they could grow up to 2-cm long in one-week period on hairy root culture medium. These results provided important addition to the knowledge of transgenic hairy root induction in the *Vitis* species.

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Multifactorial analysis of fungal tolerance in genetically modified grapevine plants: defining prototypes for commercial purposes

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Botrytis cinerea is a necrotrophic fungus that infects a large variety of horticultural crops producing "grey mold" disease and significant economic damage. Chitinases are enzymes that catalyze the hydrolysis of sugars and in fungi have a role in the growth of hyphae and differentiation. In plants, which lack chitin, these enzymes form a defense system against fungal pathogens. A 42 kDa chitinase has been purified from *Trichoderma atroviride* (ex *T. harzianum*) and its gene has been widely used in genetic transformation of various plant species leading to the generation of genetically modified (GM) plants that show tolerance against *Botrytis* infection. We have previously developed several GM grapevine *Thompson Seedless* plants and released them into a biosafety field. After three seasons of evaluations (2006-2008), the statistical analysis of the results in leaf-challenges by *Botrytis* allowed the identification of a select group of plants with a resistant phenotype. Within these lines, those bearing fruits were further analyzed for their ability as a botry-static agent in inhibition-of-fungal-growth experiments on potato-dextrose-agar (PDA) dishes. Inhibition of the radial growth and changes in the hyphae morphogenesis were observed, showing that berry juices from GM plants have an important ability inhibiting the regular radial fungal growth when compared to patterns obtained in control PDA dishes, PDA+ autoclaved GM berry juices and PDA + non-GM control juices. Results indicate the presence of a thermo-labile component in GM berry juices that inhibits growth of *Botrytis*.

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Phenotypic evaluation of 'Thompson Seedless' grapes transformed with *At*NHX1 growing in hydroponics and potted soils

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Overexpression of vacuolar Na^+/H^+ antiport *At*NHX1 has been reported to improve plant salt tolerance**.** We produced transgenic plants of *Vitis vinifera* cv. 'Thompson Seedless' via *Agrobacterium tumefaciens* carrying the gene encoding for *At*NHX1 under the control of the constitutive promoter CaMV35S. Independent transgenic lines with improved tolerance of salt stress were selected in first round evaluations. The objective of this study was to analyze the response of the selected lines to NaCl stress in hydroponics and potted soils. Untransformed and transgenic plants were acclimated to greenhouse conditions and grown hydroponically using Long Ashton nutrient solution. In the salinity treatments, NaCl concentrations were increased stepwise by 25 mM every 10 days up to 150 mM. In addition, untransformed and transgenic plants were grown in a mix of 7-3 grape pomace-sand soil and watered with Long Ashton nutrient solution with and without increasing concentrations of NaCl. Analysis of plant growth variables confirmed the improved tolerance of the transgenic lines, which showed higher shoot length, leaf number, leaf area, and fresh and dry weight of shoots and roots. However, their better performance was not correlated with higher Na accumulation in root and/or shoot tissue compared to the untransformed controls. Na and Cl tissue contents were higher in plants grown in hydroponics; and roots accumulated 1.5-2.5 times more NaCl than aerial parts.

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Characterization of grape cultivars through Expressed Sequence Tag Polymorphism (ESTP)

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The Brazilian cultivars 'Maximo IAC 138' (a hybrid of 'Shiraz' and 'Seibel' developed by Dr. Santos Neto in the Instituto Agronômico de Campinas/IAC, SP, in 1946), 'Rainha' and 'Madalena' were characterized through Expressed Sequence Tag Polymorphism (ESTP) and restriction sites. The foliar DNA was extracted using CTAB. The PCR was done using first with eleven and then thirteen primers to find EST-SNPs (single nucleotide polymorphism) in the grapevine genome. The amplicon sequencing was done after EXO-SAP purification, using the Big Dye v. 3.0/ ABI PRISM 377 from Applied Biosystems, with three replicates. The sequences analyzed with Sequencher 3.1 (Gene Codes Corporation) were compared using the Bioedit v.7.05 and analyzed *in silico* for specific restriction sites (http://www.restrictionmapper.org/). It was observed that one primer named 28 differentiated 'Rainha' from 'Máximo' in three regions, while another primer, 126, differentiated 'Madalena' from 'Rainha' and 'Maximo' in two regions and 'Maximo' from 'Madalena' in a different site. The primer 136 differentiated 'Madalena' from cultivars 'Maximo' and 'Rainha'. The other primers seem not to have amplified sequences with specific restriction site differences among cultivars. Through analysis "in silico" we observed that with primer 136, the SduI enzyme differentiated 'Rainha' and 'Maximo' from 'Madalena' whereas with primer 28, the IMx enzyme differentiated 'Maximo' from 'Rainha'. The analyses carried out using the studied primers allowed the detection of sequences with specific restriction sites and consequently, the differentiation of' Maximo', 'Rainha' and 'Madalena' cultivars**.**

P-59

Phenotypic divergence among grapevine accessions of the germplasm collection at IAC (Brazil)

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The recombination among divergent parents may result in favorable genic combinations allowing a better use of the additive, epistatic and pleiotropic effects, giving room for important characteristics such as productivity, quality and resistance to certain pathogens. Germplasm bank characterization as well as adequate statistical methods allow the identification of divergent parents. Therefore, this study aimed to evaluate the genetic divergence among 56 accessions of grapevine in the Germplasm Collection at Instituto Agronômico - IAC, characterized by eight quantitative traits. Multivariate analyses were used to quantify the divergence among the accessions. Euclidian distance, principal components and Tocher's clustering method were applied. The Tocher's method applied to the matrix of Euclidian distance with scores of principal components discriminated seven similar groups, with the first two comprised 69.64% and 17.86% respectively of total access. About 86.94% of the total variation was explained by the three first main components obtained with eight components analysis and 77.27% was explained by the first two main components. Thus, the multivariate analysis to study genetic diversity applied to the quantitative characters (Tocher's clustering method and principal components) agreed among themselves, being efficient for analysis of the genetic diversity of germplasm of grapevine at IAC, which can be used for guidance in future crosses for breeding of this species. However, additional characteristics related to productivity, cycle and quality of most will be realized and added to these works of diversity.

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The Brazilian Grape Germplasm Bank: phenology and resistance to main fungal diseases

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In recent years viticulture has reached a very important role in Brazilian fruit production, not only in temperate zones, but also as an alternative for tropical regions. These different climates require cultivars with wide-ranging production cycles. In temperate climates, such as in Southern Brazil, cultivars with different production cycles allow an increase in the harvesting period. Early grapes, demanded by growers in tropical zones such as in northeast Brazil, allow more than one annual harvest. The most important fungal diseases in Brazil are downy mildew (*Plasmopara viticola*), powdery mildew (*Uncinula necator*), anthracnose (*Elsinoe ampelina*), and bunch rot (*Botrytis cinerea* and other agents). In some cases, phytosanitary treatment can reach 30% of production costs. Genetic breeding can play a role in the development of new cultivars with different production cycles and greater tolerance to the main fungal diseases. The purpose of this work is to evaluate the phenology and disease incidence of grape accessions of the Brazilian germplasm bank in order to give support to the breeding program. For ten years, 700 accessions were evaluated and classified as very early (0.6%), early (13.9%), medium (43.6%), late (41.8%) or very late (0.3%). In the same period, 1,100 accessions were evaluated for disease resistance. The Brazilian grape germplasm bank maintains resistance sources to the main fungal grape diseases which occur in the country. However, resistance to downy mildew and anthracnose are less widespread in the studied sample. More information about the Brazilian grape germplasm bank can be found on the web at http://www.cnpuv.embrapa.br/prodserv/germoplasma /. These results assist in the development of new grape cultivars, in order to give support to the evolution and expansion of Brazilian viticulture.

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Grepevine variety determination from herbarium and arheological specimens

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Genotyping old grapevine samples, in particular variety identification via microsatellite profiling is still far from being routine. The successful identification depends on several factors, the age of the investigated material being the most obvious one. In addition, the amount and integrity of DNA depends on conditions of samples storing. Contamination and fragmentation processes make the isolated ancient DNA (aDNA) a rather difficult template for PCR amplification. Three old grapevine samples were investigated in this study. Two samples were taken from approximately 90-years old herbarium of cv. Tribidrag, a Croatian autochthonous grapevine variety not anymore present in the germplasm collections and one was approximately 2000 years old underwater archeological grapevine specimen (woody parts). Several different approaches of DNA isolation were tested. However, the use of a commercial plant DNA isolation kit showed to be the best choice in terms of yield and prevention of possible contamination with temporary grapevine DNA. The initial attempts to amplify standard grapevine microsatellite loci by standard PCR protocol failed, most likely due to low copy number of template DNA. Therefore, the whole genome amplification (WGA) kit was successfully applied to overcome this problem. The WGA approach worked in case of one herbarium sample and the archeological specimen. DNA fragments ranging between 100 and 500 bp were cloned and sequenced. While the isolated DNA from the underwater archeological sample was proved to be from different origins (not from grapevine), indicating contamination of the specimen, the six sequenced clones from the herbarium specimen corresponded to six different grapevine chromosomes. Therefore, we used the WGA-processed herbarium sample and managed to PCR-amplify the VVS2 locus, thereby demonstrating the proof of principle. Eventually, we successfully genotyped the same template using six standard microsatellite loci (VVS2, VVMD5, VVMD7, VVMD27, VvZAG62 and VvZAG79). The obtained SSR profile was identical to Zinfandel/Primitivo/Crljenak Kastelanski, except for the VVS2 locus which was homozygous (141/141) instead of heterozygous (131/141). In the light of these results we hypothesize on the origin of Zinfandel as an old Croatian variety under the historical name Tribidrag, which was being used in documents dating as late as 15th century.

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Conservation, characterisation and management of grapevine genetic resources: the European GrapeGen06 project

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The genomic revolution have been the starting point for the renewal of attention on the extent of natural occurring variation in many plants species. In this regards, the use of genetic diversity in grape may greatly improve our understanding of quantitative economically important traits. Genetic resources for grape are very numerous, in particular in Europe, but the exact extent of diversity is still difficult to know. In the scope of the European GrapeGen06 project, research institutes and grapevine collections from 17 countries of Europe, Caucasus and North-Africa are working together for the characterization and management of grapevine genetic resources, including wild accessions of *Vitis vinifera* subsp. *sylvestris*. The initial work consisted of inventorying and describing the varieties in the partners' collections, using standardized morphological, agronomic, and molecular descriptors, and to link the information into a unique European web-database. The project is in particular focusing on old autochthonous varieties. The database website, numbering more than 25000 accessions, is open for consultation, with different levels of access that allows in-dept searches but also confidentiality, according to the user needs. At the end of 2010, the fingerprint data will also be online, so that other collections or professionals can check their own varieties against the referenced varieties to verify names and true-to-typeness. The final objective of the European project is to promote an optimized conservation scheme of the *Vitis* germplasm, involving ex-situ, cryo- and on-farm conservation, so that the resources are permanently maintained, easily accessible and field-tested in a pertinent

agricultural context. This network of resources will also provide plant material as a basis for biotechnology, genomic and breeding research.

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Genetic structure and linkage disequilibrium in four *Vitis* species

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Genome-wide association (GWA) genetics is potentially of great interest in grapevine to much more rapidly and efficiently find the main loci (QTLs) and alleles involved in the variation of traits of interest to breeding, compared to searches based on individual segregating progenies. Knowledge of the structure of genetic diversity and linkage disequilibrium (LD) is a necessary prerequisite to design association genetics studies. *Vitis vinifera* genetic diversity and structure are already well characterized, but LD in this species has not been thoroughly estimated yet. Other *Vitis* species are a large potential source of resistance to biotic and abiotic stresses for introgression into *V. vinifera*, but relatively few genetic resources are available in germplasm repositories and the genetic diversity of these species is hardly known. The objectives of the DLVitis project (ANR 2009-2011) are to assess genetic structure and linkage disequilibrium in *V. vinifera* and 3 American species (*V. riparia, V. cinerea, V. aestivalis*). For *V. vinifera*, 3 samples of 93 cultivars were defined, based on available SSR data, by minimizing structure and kinship and maximizing genetic distances. An additional sample of 93 accessions of wild *V. vinifera* (subsp. *silvestris*) was also defined. For each American species, 150-200 seeds were collected *in natura* last autumn. Genetic diversity and structure will be assessed this year using SSRs, and a sample of 93 accessions will be defined for each species. Four genomic regions of 2 Mb each were chosen to estimate LD between specific SNPs in *ca.* 1 gene out of 3 in each sample of 93 individuals described above. Novel estimates of LD taking structure and kinship into account were developped and will be presented. The first results obtained in *V. vinifera* in 2 genomic regions showed differences in the rate of LD decay between samples and regions, suggesting past occurrence of differential selection. The consequences of these results for the design of GWA studies will be discussed.

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Genetic structure in a large representative sample of cultivated and wild grapevines

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The genetic structure of a collection of more than 2900 unique genotypes representative of both the cultivated and the wild grapevine gene pools was studied with the help of nuclear neutral and non-neutral molecular markers. The subgroups obtained through complementary clustering methods were matched with their geographic origins and their phenotypic characterizations. The main clustering obtained with microsatellite markers on the larger genotype dataset, was confirmed by the SNP variation of 80 genes of reference, chosen as the most informative ones out of a total of 960 genes, re-sequenced on a subset of representative genotypes (core-collection). The partition of diversity of both neutral and non-neutral markers pointed to patterns that could be related to historical processes of domestication, selection and breeding, and to geography, from ancestral groups derived from opposite regions of the grapevine distribution area, down to a number of subgroups linked to specific local uses and grape features, finally merging into a family level. Within the cultivated compartment, the three most significant subgroups corresponded to the wine grapes from the Western Europe, the wine grapes from Balkans and Central Europe, and the table grapes from the East. A further subdivision evidenced family or sub-regional structures, such as the groupings of grapevines from Maghreb, from the Italian and the Iberian peninsula, from Russia and the Caucasian region, or the Muscat, the Savagnin and the Pinot-Gouais families. The study of the relationships with the wild compartment revealed that the cultivated gene pool was derived from two separate wild grapevine gene pools, one wild pool from the East that contributed to the table grapes, and a second wild pool from West Europe that contributed to the wine grapes. Presenting the more complete dataset to date, this work allows researchers to precisely account for genetic structure when sampling diversity within unstructured groups, as it is required by modern methods of genome-wide association genetics. It also adds a layer of information to the already documented national grapevine collection of Vassal, France, confirming its role as a reference for grapevine genetic studies.

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Plastid DNA sequence diversity in a worldwide set of grapevine cultivars (*Vitis vinifera* L. subsp. *vinifera*)

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DNA sequence diversity was investigated at two plastid regions (the *trnH-psbA* intergenic spacer and the *rpl16* intron) in a geographically diverse group of 113 cultivated grape samples. This group included 40 samples from the Republic of Georgia, home to over 500 grape cultivars and the earliest archaeological evidence of grape domestication. The greater Caucasus region in which Georgia lies is widely believed to be the area in which grape domestication began, and the study of genetic diversity in this region is viewed as key to understanding grape domestication in general. Four plastid haplotypes are evident in the 113 samples, and are designated by their character-states at each of the 3 polymorphic positions: (AAA)–23 samples, (ATT)–29 samples, (GTA)–34 samples, and (ATA)–27 samples. The AAA haplotype was only observed in Georgian samples, and these 23 "Rkatsiteli" group cultivars originate mostly from eastern Georgia. Contrast this group with the nine Georgian cultivars (23%) of the "Chkhaveri-Pinot noir" group (GTA), most of which are cultivated in western Georgia near the Black Sea coast. The observation that the Georgian cultivars exhibited both unique plastid DNA variation (the AAA haplotype) and all other observed plastid haplotypes is consistent with previous studies that have observed both unique and high levels of genetic variation in wild grape (*V. vinifera* subsp. *sylvestris*) in the greater Caucasus region.

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Analysis of grape rootstocks by microsatellite markers

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The aim of this work was to determine the SSR profile of 96 *Vitis* accessions (mainly rootstocks) at 19 loci and to find genetic relatedness between them. Molecular markers used in the last 30 years are not effected by the environment, giving a good tool for determination of genetic distances. Based on our former experiences SSR analysis was carried out on 96 grape accessions, mainly rootstocks. DNA was extracted from young leaves. Analyses were carried out using 19 microsatellite loci. The loci were selected to cover all of the linkage groups to get more exact impression of the genome of the grape rootstocks. Results of SSR analysis and a dendogram showing genetic relatedness were constructed. Based on the results it can be established that most of the loci showed appropriate polymorphism. High similarity was detected between the clones of T5C and TK5BB. Strong differences were detected between the so called “clones” of T5C and TK5BB, suggesting, that they are not real clones, but different genotypes with highly similar morphological features. The *Vitis sylvestris* accessions showed close relatedness with the *Vitis vinifera* L. varieties.

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Analysis of pinot varieties by microsatellite markers

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Molecular markers are widely used because of the wide range of applications. The identification of the varieties and clones of Pinots by microsatellite method according to the literature, present difficulties Hocquigny et al (2004) carried out SSR analysis between Pinots. Polymorphism analysis between 'Pinot' clones revealed that 65% shared the most frequent genotype. Moreover, the variant clones showed at least 96% similarity with this genotype. Based on our former experiences 7 Pinot gris, 4 Pinot noir clones and Pinot blanc were analysed in 16 (VVS2, VMC5E9, VMC3D12, VVIM10, VMC5G8, VMCNG1E1, VMC1F10, VMC2H4, VMC8A7, VMC7G3, VVMD28, VrZag21, VrZag79, VMC1C10, VrZag25, Scu06vv) microsatellite loci. A dendogramm was constructed using Jaccard index for the estimates of genetic similarity between pairs, and average linkage for clustering.
Based on our results, it can be established, that the Pinot clones all showed high similarity. The Pinot gris clones bred in Badacsony, Hungary (B. 10, B. 10/5, B. 10/10) formed a group and showed the highest similarity with Pinor gris 34 from Romania. The other Pinot gris clones formed another group with Pinot noir C-162. These clones all originated from western Europe (Germany, France). These genetic differences could be traced back to the different geographical origin of the different clones.

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Italian wild grapevine: a state of the art on germplasm and conservation in 2010, the Year of Biodiversity

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Vitis vinifera sylvestris is the wild progenitor of cultivated grapevine. Wild grapevine populations are part of the European woodlands. In some countries wild grapevine is considered on the brink of extinction. In 2006 the Italian Ministry of Environment funded a research project aiming to the creation of a germplasm collection of wild grapevines pooled from Italian existing populations. The project involved the Milano University and the Cinque Terre National Park which is characterized by an agricultural landscape made up of terraces cultivated once almost exclusively by vines held up by stone walls. Today most of the terraces are still present but are at constant risk of landslides due to their abandonment. Cinque Terre was designated a UNESCO world heritage site for its landscape agriculture and for the need of preservation of its cultural, environmental, local and historical values. One of the purposes of the collection inserted in this frame is to recover a part of the abandoned terraces. Moreover the experimental vineyard has both the characteristics of *in situ* and *ex situ* collection and is dedicated to researchers interested in the study of the genetic structure of wild grapevine, both for population genetics and for studies regarding gene diversity (in the wild and cultivated compartment). For this reason 600 samples representative of Italian wild grapevine populations widely dispersed in woodlands have been collected. At this time 300 among these are already in the collection and have been genotyped at 20 SSR loci both, to have a correct fingerprint of all the accessions and to avoid mistakes during samplings. Based on molecular data and on the comparison with previous censuses some considerations about the genetic structure and conservation of Italian wild grapevine populations and their level of introgression with Italian cultivated germplasm have been made. In the next years the collection will be monitored to collect data regarding phenology and possible pest tolerance; and to improve knowledge on genetic traits differentially expressed in the two subspecies. In the year devoted to biodiversity, this collection has the intent to highlight two important features: one related to the safeguard of an harsh land imprinted and tamed by agricultural activities and human labour; the other devoted to the understanding and use of the genetic resources present in the wild grapevine compartment evolved in a natural environment untouched by human activities.

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'Ribolla Gialla' from north eastern Italy, 'Rebula' from northern Balkans and 'Robola' from Ionian islands: do they belong to the same population variety or are they genetically different?

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'Ribolla Gialla' is an extremely old grape cultivated in the Italian region Friuli-Venezia Giulia in the provinces of Gorizia and Udine. Once part of the Venetian Republic, Friuli Venezia Giulia has been for long time an important step along the Mediterranean spice route from the Byzantine Empire to the trading centre of Venice. During the Middle Ages, travelers passing through this area are supposed to have brought grapevines from Balkans and Anatolia. Referring to historical information and reports Ribolla has been the leading quality wine used as a representative sign to honor famous figures of the ages. Known in Friuli since Roman Empire, for some authors, Ribolla would correspond to the Roman famous 'Avola' wine. Others believe that the Roman 'Punicinum' used Ribolla as its base wine. After the splendors and reputation gained by Ribolla wines in the past centuries, the beginning of the 1900s marked a difficult period for the wine, with a very modest reputation. And, in the '60s of the past Century the interest in the cultivation of Ribolla variety decreased, in the Italian region, leaving place for the cultivation of Tocai Friulano, Pinot Bianco and Grigio, Sauvignon, Traminer and Riesling. Despite the historical documents reporting the longstanding tradition of Ribolla cultivation and winemaking in Friuli-Venezia Giulia the origin of this variety is still questionable. The neighbor Regions Slovenia and Dalmatia have under cultivation a white berried wine-grape variety called Rebula, and in Greece (in the Ionic islands of Kefalonia) we have white wines produced with a variety named Robola. The aim of this work is to study the genetic structure of these 3 groups of white varieties and verify the existence of genetic relationships linking these accessions sharing the same name. For this purpose 20 SSR loci were tested to fingerprint 30 Ribolla-Rebula-Robola accessions uniformly distributed in the 4 areas of cultivation. Data obtained proved the existence of some synonym accessions and relationships between Ribolla-Rebula and Robola accessions. Basing on these results, indigenous varieties belonging to the same area of cultivation of the Ribolla-

Rebula-Robola group were genotyped at the same SSR loci and some interesting results were obtained, helping to develop hypothesis about the place of origin of this group.

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Proteomic analysis among different 'Aglianico' ecotypes

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Molecular markers are extensively used for the identification of different vines. Among them, one of the techniques of major success is based on the analysis of the Simple Sequence Repeats (SSRs). Although they are useful in discriminating among different varieties, it is unusual that they can distinguish clones deriving from the same vine and there is a strong request for devices that could achieve this purpose. In this view, it could be interesting to evaluate the potential of proteomics. Using this approach, for instance, it was possible to define the biodiversity of six ecotypes of *Arabidopsis thaliana*. Moving from these considerations, three ecotypes of cv. Aglianico (Taurasi, Taburno and Vulture) were compared. They look identical but give rise to wines that are characterized by different qualitative features. Through the use of 20 SSRs markers, it was demonstrated that they belong to the same cultivar because it was not possible to find any differences. The proteomic analysis was conducted comparing the 2-DE patterns of berry exocarp in order to set up a method useful in searching for distinctive traits. On the basis of previous studies that underlined the changes of the proteome of this tissue during maturation or driven by the environment, the analysis was performed considering two moments of maturation during the years 2008 and 2009. In order to deal with the high number of data and to sort the variability among them, gels analysis, after their alignment, was carried out through multivariate statistical techniques such as Principal Component Analysis and Linear Discriminant Analysis. The application of such approaches allowed to distinguish the three ecotypes and to isolate the proteins more responsible of the discrimination models, then characterized by mass spectrometry. These results confirm the effectiveness of this approach integrating proteomics and multivariate statistics for the study of intravarietal diversity.

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Viticultural performance of some 'Cabernet Sauvignon' clones

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Ten clones of *V. vinifera* L. 'Cabernet Sauvignon' from different origins (Italy, clone R5; Chile, clone ISV117; California, clones VCR8 and ISV2; France, clones 191, 341, 338, 169, 685; Argentina, clone ISV105), were grafted on *V. Berlandieri* x *V. riparia* S.O.4 rootstock and grown in pots to compare vegetative (phenological phases; shoot growth; internode length), productive (bud fertility, grape yield, mean cluster and berry weight) and qualitative (soluble solids, pH, titratable acidity, anthocyanins, polyphenols, *trans*-resveratrol) parameters. Meteorological conditions were recorded as well. According to analysis of variance and discriminant analysis the clones have been grouped in four clusters irrespective of their origin.

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The software for a "Universal Grapevine Database"

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Software for a "universal grapevine database" has been developed to support the characterization of grapevine biodiversity. In this database, registered users may submit and manage their own data at any time, and the introduced data may have three different levels of visualisation: private level (the data are visible only to the submitter), middle level (the data are visible to all other submitters), public level (the data are visible to all public users). Only the data approved by a specific scientific committee may be elevated to public level. When a submitter introduces microsatellite data into the database, the application allows a specific standardization procedure based on some specific selected accessions called 'system accessions'. The main classes of data represented in the database are the 'grapevine variety', the 'ampelographic-ampelometric and phenological-productive descriptors' (as reported in the second edition of the OIV descriptor list for grape varieties and Vitis species), the 'microsatellite profile', and the polyphenol and aroma profiles. Several search options have been implemented: a search by variety and other general parameters, a search by ampelographic and ampelometric parameters, and a search by microsatellite profile. In the microsatellite profile it is also possible to search by range and by a particular standardization procedure. The application has been implemented using the most recent database software and languages, so it is flexible and dynamic especially as with regard to the addition of other classes of data, like new type of descriptors and molecular markers. The application is on the web at http://www.vitisdb.it and at present it is adopted for the 'Italian *Vitis* Database', managed by the "*Vitis* Database Working Group".

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Molecular characterization of the grapevine germplasm collection held at the Fondazione Edmund Mach

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The grapevine germplasm collection maintained by FEM-IASMA was set up in the 1980s from a small core of pre-existing varieties, which was supplemented with material from various international sources, as well as minor and wild grapevine varieties from Italy. It currently includes about 2,000 accessions of *Vitis vinifera sativa* and several hundred individuals of *V. vinifera sylvestris*, as well as other species of the genus *Vitis* and hybrids. All accessions are planted in the same locality in five replicates on Kober 5BB rootstock and trained on the Guyot system. Growing scientific interest in the genetic diversity of grapevine, together with the need to improve the organization of materials and streamline management costs has given rise to a project aimed at characterizing all the conserved germplasm. In an initial molecular analysis, one plant of each accession was typed at ten microsatellite loci (SSR). Comparison of the profiles obtained allowed the genotypes present to be identified and hence the number of accessions to undergo further investigation to be reduced. The extension of genotyping to 12 other SSR loci confirmed the consistency of the collection's gene pool. Using assignment experiments, comparisons with international databases and ampelographic verification, grape varieties with the same SSR profile are attributed to cases of synonymy or denomination errors. The most interesting phenotypic variations amongst plants with an identical SSR genotype will be selected for conservation. The set of accessions with a non-redundant genotype is the collection's main source of genetic diversity. Regarding this material, intense genotyping of hundreds of SNP loci is underway in order to identify appropriate core collections for future association studies. Some of the characteristics emerging from this study and other potential evaluations of the collection's contents will be presented.

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Application of SSR markers for must and wine varietal detection

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The control of wine quality and authenticity is becoming a necessity because customers have the right to be informed about the product they buy and consume. On the other hand, the prospect of adding grape varieties beyond those permitted by law can lead to fraudulent practices in wine industry. DNA microsatellite (SSR) markers are powerful tools used for cultivar identification with genomic DNA, which is easily extracted from leaves. However, it is rather difficult to obtain high quality DNA from must and wines samples because of its low concentration in the samples, the low reproducibility of DNA analysis and DNA degradation during vinification processes. Therefore, an optimized DNA extraction method from musts and wines was developed. Experimental microvinifications were done from six white cultivars and five red cultivars and used for the experiments. The results indicated that suitable DNA extraction methods for must and wines leading to high quality DNA and PCR amplification for short target sequences, such as SSRs, are indispensable to conduct DNA profiling using must and wines samples. Thus, cultivar identification could be performed based on analysis of the nuclear SSRs recommended by OIV (VVMD5, VVMD7, VVMD27, VvZAG62, VvZAG79, and VVS2). Therefore, the analysis of SSRs markers can be used for must and wines identification for quality control, certification, and traceability purposes.

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Variation of berry polyphenolic profiles of USDA *Vitis vinifera* germplasm

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Polyphenolics are an important class of secondary metabolites with significant benefits to human nutrition and health. As a part of the effort to characterize USDA *Vitis* germplasm, we evaluated the polyphenolic profiles in the berry samples of 347 *Vitis vinifera* cultivars, maintained at the USDA *Vitis* Clonal Repository at Davis, CA, for two successive years. A total of 36 phenolic compounds were identified via HPLC-MS and quantified by HPLC-DAD, including 16 anthocyanins, 2 hydroxybenzoic acids, 6 hydroxycinnamic acids, 6 flavonols and 6 flavanols. The total anthocyanins ranged from 0 to 5.12 mg/g FW in 2008, and from 0 to 5.02 mg/g FW in 2009. The means of total anthocyanins in red cultivars were 1.47 and 1.49 mg/g FW, respectively, in the two years. Malvidin 3-*O*-glucoside and malvidin 3-*O*-(6-*O*-coumaryl)-glucoside were the most abundant anthocyanins, accounting for 60% of the total anthocyanins. Anthocyanin concentrations were significantly higher in wine grapes than in table grapes in red cultivars. Hydroxycinnamic acids ranged from 0.01 to 0.93, and from 0.01 to 0.87 mg/g FW in the two years, with caftaric acid being the most abundant (accounting for 70% of the total). The total hydroxycinnamic acids in wine grapes were significantly higher than in table grapes. Flavanols ranged from 0.004 to 1.42 and 0.04 to 1.92 mg/g FW, respectively, in the two years, with procyanidin B1 being the most abundant (accounting for 50% of the total). Flavonols ranged from 0.01 to 0.2 in both years, with quercetin 3-*O*-glucuronide and quercetin 3-*O*-glucoside being the most abundant. Hydroxybenzoic acids were the least (<0.02 mg/g FW) among all phenolic compounds, but the mean concentrations were higher in wine grapes than in table grapes. Significant positive correlations were found between anthocyanins, hydroxybenzoic acids, hydroxycinnamic acids, flavonols, and flavanols. Principal component analysis indicated that there was significant variation in all phenolic compounds, and the first two principle components (PCs) accounted for more than 80% of total variance. Malvidin 3-*O*-glucoside and malvidin 3-*O*-(6-*O*-coumaryl)-glucoside, petunidin 3-*O*-(6-*O*-coumaryl)-glucoside, and petunidin 3-*O*-glucoside were major contributors to PC1, and caftaric acid was an important contributor to PC2.

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Aestivales (Planchon), American Native Grapes: Phylogenetics and Use for Breeding of High Quality Wine Grapes for Southeastern United States

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Cultivars of '*Aestivales'* are the only American native grapes with remarkable juice and wine color stability. The native grapes of the Americas have provided valuable germplasm for improvement and development of cultivated commercial grape genotypes for fresh fruit, wine and processing throughout the United States and Canada, where *Vitis vinifera*, or "old world grape", can not grow. Also they have qualities for wine longevity and very pleasant "mouth feel". Members of the '*Aestivales*' group including *Vitis aestivalis* Michaux, are found in the eastern and central United States, from New England to Florida and from Wisconsin to Texas. The close proximity of related species and many variants to *Vitis aestivalis* has created confusion among taxonomists trying to classify grape species. The aim of this research study was to define the phylogenetic relations among the grape species and subspecies listed under the '*Aestivales'* Planchon group via data mining in the existing North American grape germplasm collections, and specifically expressed in the members of the group DNA microsatellites for using them in breeding programs and grape improvement. DNA isolation and quantification of nine *'Aestivales'* accessions, evenly distributed throughout their area of natural habitat was completed. Microsatellite specific PCR amplification products were obtained in nine out of ten microsatellite markers from *V. riparia* which were previously and successfully used for grape identification. The amplicons were subjected to fragment analysis and then analyzed using the software STATISTICA, version 4.5 and a dendrogram was generated. Results found using the Applied Biosystems Sequencer (ABI) showed two distinct groups, the first group had accessions acquired from the south and midwest while the second group had accessions acquired from the north. Thus indicates that there was a clear delineation between the origin of the accessions and proximity of their relationship in the dendrogram. As a spin out of the "pedigree" research of "Cynthiana" var. the comprehensive germplasm collection of *Aestivales,* Planshon native grapes was established and was used as genetic pool for further directed hybridizations. More than 100 of the new selections were planted and are currently under evaluation for desirable quality characteristics.

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Morphological and molecular identification of Maragheh grapevine cultivars

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Maragheh, Iran, has a long history in viticulture, and grapes (*Vitis vinifera, L*) are one of the most important commercial fruit crops grown and used for raisins, juice and fresh fruit. Most of the cultivars are unknown to growers and breeders however, and their genetic characterization is necessary to the growing and breeding programs. The goal of this study was to identify the cultivars planted in Maragheh, based on morphological and molecular information, for planning the growing and breeding programs. Fifty cultivars and their morphological traits were characterized through ampelography and 14 SSR primers were used to confirm the results. Results showed significant differences among all of the studied cultivars based on morphological characteristics. The SSR primers chosen for this study discriminated 30 different genotypes among the 50 analyzed cultivars.

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Genetic diversity in Maragheh grapevines pollen traits

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The grapevine (*Vitis vinifera, L*) is cultured widely in Maragheh making it one of the most important commercial fruit crops. Most of the cultivars are used as rasin, juice and fresh fruits crops. However, pollination is the main factor affecting fruit set, furthermore, knowledge about pollen traits of cultivars is necessary to plan the breeding programs. This study accomplished to investigate pollen traits of the main grape cultivars/ genotypes planted in Maragheh, for identifying genetic diversity of their pollens to use in the future different breeding programs. Pollens of 10 cultivars/genotypes gathered and cultured in the *in-vitro* medium contained Sucrose, Boric acid and Agar. Experimental design was completely randomized with 10 treatments and 4 repeats and data analyzed with SAS software. Main pollen traits are important in breeding programs, were studied by light microscope weekly in 10 weeks including pollen viability, longevity, germination percentage and tube growth. Results showed significant differences among all of the studied cultivars/genotypes and cultivars clustered based on studied pollen traits for using in the different breeding programs.

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Studies on an excellent, early-maturing bud sport of 'Kyoho'

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The 'Kyoho' grape is a European-American hybrid cultivar with strong disease resistance and wide adaptability. Fortunately, we found an excellent bud mutation of 'Kyoho' from orchard by selection. The bud mutation matured seven days earlier than 'Kyoho' in phenology for five consecutive years. The berry weight averaged 12-15 g. The berry size and the content of soluble sugar, pulp firmness, bearing pull force were also improved compared to those of 'Kyoho'. Using the bud mutation as scion, the appropriate rootstocks were determined from those widely used in the south of China. To evaluate the genetic relationship between the bud mutation and its maternal parent 'Kyoho', 42 grapevine simple sequence repeat (SSR) markers were used to amplify DNA of 'Kyoho' and its bud mutation. These microsatellites are highly polymorphic and have been considered suitable for assessing variation among grapevine collections. The results showed the genetic similarity from these SSR markers is 97.6% and a pair of SSR primers VrZAG67 can be used to distinguish bud mutation from 'Kyoho'.

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Generation of a new polyploidy grape cultivar by a new strategy that combines crossbreeding with colchicine-induced mutation

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A new polyploid grape cultivar was generated by a new strategy that combines traditional crossbreeding with a chemical-induced mutation method. Germinated diploid hybrid grape seeds were treated with different concentrations of colchicine at 20^0C during day and 25^0C at night for a variety of time periods. Seedlings were examined morphologically for the initial mutation screening in the field. Comparison between seedlings from hybrid seeds and self-pollinated seeds indicated that the rate of deformity is 3.54% and 25.8% for hybrid and self-pollinated seeds, respectively. Analysis by tip-end slicing method to measure the size of the L_1, L_2, and L_3 of the meristem demonstrated a significant difference between diploid and tetraploid cultivars. Results from chromosome counting in root tips indicated that tetraploid descendants are 2n=4x=76 while the diploid parents are 2n=2x=38. Based on the size and shape of pollen and stomata and number of chloroplasts in guard cells, it was concluded that the new grape cultivars selected in this study have similar characteristics to 'Black Olympia' which is a well-known tetraploid cultivar. The data from pollen viability and fruiting rate also confirmed that cultivars we selected are tetraploids. By using this new strategy, we successfully generated several new polyploid cultivars that produce fruit earlier with high quality in terms of fruit size, seedlessness and taste.

P-81

Effectiveness of different culture media on the success of embryo rescue among different crosses of seedless grapes

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Effects of different culture media were tested on embryo rescue among three different cross combinations of seedless grapes. Here ER, Nitsch, WP, half strength of B_5 and 1/2MS media were used. The best medium for ovule development was ER＋6-BA 0.5 mg/L among all the cross combinations. However, for rescued embryo regeneration and subculture, the most suitable media varied among different cross combinations. The best media for seedling establishment in 'Flame seedless' × 'Centennia' were 1/2 MS for embryo germination, 1/2 MS + IBA 0.1 mg/L for seedling growth, 1/2 MS + IBA 0.1 mg/L for subculture, respectively. The best media for 'Otilia' × 'Venus' were 1/2 MS + IAA 0.1 mg/L for embryo germination, 1/2 MS + IAA 0.1 mg/L for seedling growth, and half strength of B_5 + IAA 0.125 mg/L for subculture, respectively. The best media for 'Venus' × 'Himrod' were 1/2 MS for embryo germination, WP + 6-BA 0.1 mg/L for seedling growth, half strength of B_5 + IAA 0.125 mg/L for subculture, respectively.

P-82

Polyploid induction of mutation by using colchicines on tube seedlings of Victoria grape

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The unigemmius stem-segment of the tube seedlings of Victoria grape were soaked 24 hours and 48 hours by using colchicines aqueous solution of 0.05% and 0.1% concentration. The results showed that the best way for reduplicative efficiency was the third and fourth buds treated for 48 hours in 0.05% colchicine and 24 hours in 0.1% colchicine, the doubling rates of primary generation cells were 33% and 31% respectively; The polyploid cell rate could increase about 10% by subcultured the chimeras a generation. The polyploid cell rate could reach 75.8%-96.6 % by subcultured the chimeras four to six generations. Through root tip cell chromosome observation that it was a little effect to the division cycle of root-tip cells in terms of the tube seedlings with or without illumination in short term., and the optimal sampling time of chromosome for observation was at 9:30 am.

P-83

Some early maturing table grape cultivars with muscat flavor realeased by Beijing Institute of Forestry and Pomology

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In China, people like to eat the grapes with muscat flavor, but in the market of Beijing areas, the early ripening cultivars are very limited. A breeding program was started in the Institute of Forestry and Pomology 50 years ago aiming to get early ripening table grape cultivars with muscat flavor and adaptation for local areas. Since that time approximately 150000 seedlings have been grown from the 2500 crosses, the germplasm used were the traditional cultivars of *Vitis vinifera,* Muscat of Hamburg was chosen as the female parent for its muscat flavor, Pearl of Csaba and Jingzaojing were chosen as the male parents due to their muscat flavor and early ripening characteristics. To date seven new table grape cultivars have been released to the public, Xiangfei, maturing at the first to second week of July with yellow to golden color. Ruiduxiangyu, very crispy and sweet, berry size over 18mm. Emerald with yellow green skin color and good resistant to disease. Agate early, maturing at the third to fourth week of July and good resistant to cold. Purple Pearl, purple skin color with good keeping quality. Muscat Early, similar to Muscat of Hamburg but maturing 3 weeks earlier, and Muscat Angel is seedless with purple reddish color, all of them inherited the flavor of Muscat Hamburg and maturing date was early than the comparative cultivars. These cultivars have been used in the Beijing grape growing area and spread to some northern provinces.

P-84

Progress in grapevine breeding in China over a decade

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In order to meet the requirements of fast growth in the Chinese grape industry, grapevine breeding has received great attention and been threatened in China in the last ten years. There have been more than 20 units conducting active grapevine breeding programs across the country. The aspect of the effort is obvious. There have been 68 new varieties of grapevine that have been released from these programs. Among them, thirty eight are table grape, others are rootstock or for wine production. The selection of table grapevine varieties carrying characteristics such as disease resistance, early ripening, high tolerance in storage and transportation, seedlessness and having amrose-scented aroma is the main target in these breeding programs. Cross breeding is still the main method for producing new varieties of grapevine. However, other methods such as seedling selection, asexual selection, and induced mutation breeding and biological technologies have been used by more and more researchers in their work in recent years. The results are also promising. All these progresses are reviewed in the article.

P-85

New triploid seedless table grape cultivar 'Moonlight Seedless' bred in China

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'Moonlight Seedless', released in 2009, is the third cultivar from our triploid seedless table grape breeding program initiated in the early 1980s. The previous releases, 'Earlyred Seedless' (1998), 'Champion Seedless' (2003), are serving as some of the leading cultivars for the seedless table grape industry in Hebei Province. 'Moonlight Seedless' originated from the cross between the diploid 'Muscat Hamburg ' *V. vinifera*） and the tetraploid 'Kyoho' （*V. vinifera* × *V. labrusca*） made in 1991. Agrobiological properties, characteristics of bunches and berries, and productivity of 'Moonlight Seedless' were investigated. 'Moonlight Seedless' is triploid. The vines are very vigorous. It ripens in late August, at the same time as 'Kyoho'. Bunches are loose with small, seedless berries. GA3 and CPPU treatments are necessary to improve bunch and berry size. The bunches are conical, relatively compact, averaging 581g and the berries are 9.0g on average, purple black with medium crisp flesh. Soluble solid content averages 19.4%. It is very productive. The resistance of the cultivar to diseases is similar to that of 'Kyoho'.

P-86

Conferred vigour: loci but no logic

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Rootstocks can be used as efficient tools to control the development of grapevines and thus can improve the quality of the harvest. This phenomenon is known as conferred vigour and is not related to the own vigour of the ungrafted rootstock, therefore making it difficult to predict the conferred vigour of new rootstock varieties. In order to improve the selection efficiency and shorten the cycle of rootstock selection, we aim to identify the regions of the genome controlling own and conferred vigour of rootstocks to develop a Marker Assisted Selection program. An F1 population composed of 130 individuals issued from a cross between 'Cabernet Sauvignon' x 'Riparia Gloire de Montpellier' was evaluated for conferred rootstock vigour. The F1 population was grafted with 'Cabernet Sauvignon' as the scion. Four different experimental designs were used: a pot experiment with one year old plants, a pot experiment with two year old plants, a long term field experiment and a short term field experiment comparing five different scions ('Cabernet Sauvignon', 'Ugni Blanc', 'Petit Verdot', 'Muscat de Hambourg' and 'Merlot') grafted on the whole F1 population. In pot experiments and in the multi scion field experiment, plants were trained on a single stem. Vigour was evaluated by measuring the growth of the stem. Dry matter of the root system was also evaluated. In the long term field experiment, we measured the pruning weight and harvest weight. QTLs for induced vigour were mapped on the genetic map of the population. Common QTLs were detected between field and pot experiments. The multi scion experiment allowed us to find new QTLs which were not identified with 'Cabernet Sauvignon' as the scion. This adds to the complexity of this phenomenon and suggests that the interaction between the rootstock and the scion is the key to understanding conferred vigour.

P-87

Grape breeding and viticulture research programe in Iran

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Iran is a large country with more than 320,000 ha of cultivated grapevines. Several species of the genus *Vitis*, such as *Vitis sylvestris* (in west) and *V. labrusca* (in north) are native to Iran. However, they have not been brought into cultivation or for breeding. Also, There are more than 800 genotypes of grapevine include commercial cultivars and wild genotypes in Iran. Now, there are 680 genotypes of them from local collections in Takestan Grapevine Research Station in Iran. The progenies from the vine adapted to the environment of that district, and the cultivar 'Kishmesh' were thus released. In the 6th century, 'Sefid bidaneh' grapes became famous for their excellent fruit quality. However, most *vinifera* grapes could be cultivated successfully, but not in north of Iran, because of the spread of diseases. Until 2007 there aren't any identified programs for viticulture and breeding research on grape and generally, researchers worked based on regional requirements. Since, precise research program is vital for viticulture in Iran. It was decided to prepare based on 20 years future a document of Iranian agriculture development strategies. The organization of agriculture research, education and extension was responsible to do that. A team of all researchers and experts of viticulture work together for perpetration of this program. The main objects of this program are extension of cultural area, quality and quantity improvement of table grape and raisin. The program includes four sections:

1. At first stage, viticulture problems and limitations were detected in all regions of Iran and analyzed.
2. In second stage, a tree of problems was drawn. Tree drawing was based on situation and determination of problems importance that influenced instability of grapevine production in Iran.
3. Gathering of information were done from results of last research in Iran and other countries to solving problems in 3rd stages of program.
4. Tree of aims of program was drawn. This tree showed necessary works that may be done to solving problems of viticulture improvement in Iran. Based on tree of aims team tries to provide a program that includes in research requirements and time schedule of works.

P-88

‘Tankeumchu’: A mid-ripening, black-fruited table grape

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‘Tankeumchu’ (*Vitis sp.* X *Vitis vinifera*) is a new table grape cultivar that is diploid, ripens mid-season and black-fruited with large clusters (Fig. 1). It has a mean bud burst on Apr. 24, full blooming on June 1, and fruit maturity on Sep. 10. It has an excellent taste with abundant juice and soft firmness. The berry’s mean weight is 7.0 g, and mean content of soluble solids 18.0 ^{o}Brix. ‘Tankeumchu’ is more suitable to the mild climate with its moderate resistance to cold hardiness.

P-89

The usefulness of allotetraploidy and isolated embryo culture *in vitro* for obtaining intergeneric hybrids of grape

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The development of intergeneric hybrids of grape within the *Vitaceae* family was attempted by using allotetraploidy combined with isolated embryo culture *in vitro*. Plants of the genus *Vitis* (eight *V. vinifera* cultivars, 2n = 38) were crossed with those of the genera *Ampelopsis (A. acontifolia, A. cordata*, 2n = 40*)* and *Parthenocissus (P. inserta* and *P. guinguefolia*, 2n = 40). To obtain tetraploid forms, buds broken from dormancy were treated with colchicine at 0.5%. The presence of pairs of chromosomes is a necessary prerequisite for synapsis during meiosis and for gamete formation, which is required for fertility in hybrid seedlings. Plants were grown *in vitro* from embryos isolated from the green berries 40 days after the fertilization. The crossings yielded only two seedlings of 'Kharty pro Livie' x *Ampelopsis cordata* and 11 seedlings of 'Picpoule noir' x *Ampelopsis acontifolia,* because the development of intergeneric hybrids is associated with considerable difficulties due to genetic and biochemical incompatibility of different genera within *Vitaceae*, which in the first case may lead to genetically unstable plants. The dissection pattern of the leaf in the progeny was different from that of the *V. vinifera* cultivars used as the female parents (flowers with female flower parts) which had undissected leaves. This suggests quite reliably that this character was inherited from the male parents and the plants obtained are intergeneric hybrids of grape. The possibility of obtaining such forms artificially indicates that they might have arisen from distant crosses in nature in the course of natural evolution.

P-90

Genetic control of phyllotaxy phase shift in juvenile vines in a rootstock hybrid population

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Grapevine seedlings initially display spiral phyllotaxy of true leaves, then undergo a shift to alternate phyllotaxy with the production of the first lateral meristems (typically tendrils). The node at which the shift from spiral to alternate phyllotaxy occurs varies from about the 4th to about the 12th node on the vine. To investigate the genetic control of the transition from spiral phyllotaxy to alternate phyllotaxy, a population segregating for this trait was developed and screened. The population derived from four female parents and six male parents crossed in a Design 2 mating array (all female parents crossed to all male parents). The female parents were the pistillate flowered rootstock varieties 1613 Couderc, 93-5 Couderc (California clone), *Vitis rupestris* 187G, and Fercal. The male parents were staminate flowered grape rootstock germplasm, species, and species hybrid selections with diverse backgrounds, including accessions from the USDA ARS National Clonal Germplasm Repository, Davis, California (denoted with DVIT accession numbers): IAC 572, *Vitis labrusca* Y137 DVIT 1392, *Vitis* hybrid Y93 DVIT 1519, *Vitis* hybrid Q126 DVIT 1456, *Vitis* hybrid Q130 DVIT 1466, and *Vitis* hybrid R127 DVIT 1490. The species background of the male parents includes *V. labrusca*, *V. mustangensis*, *V. riparia*, *V. tiliifolia*, and *V. rupestris*. Seedlings from controlled crosses were grown in individual pots in a greenhouse with artificial illumination to provide 24 hours day length throughout cultivation. The node number of the first observed lateral meristem was recorded; the goal was 50 seedlings per population for each of 24 populations, although some populations showed poor seed germination. The transition from spiral to alternate phyllotaxy does not appear to be under simple genetic control, but there is significant variation from family to family in this trait. It should be possible through selection to increase the node number of the transition from spiral phyllotaxy, which is lateral meristem free, to alternate phyllotaxy. Reduced tendril counts on rootstocks would increase the efficiency of grape rootstock cutting production in nurseries.

P-91

Drying rate of fresh berries from natural dry-on-the-vine (DOV) grape germplasm.

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The California raisin industry has turned to mechanical harvest to increase production efficiency. Fruiting canes of raisin cultivars must be cut to induce the fruit drying process. Grape germplasm that has its fruit dry-on-the-vine (DOV) without having their canes cut have been identified. Identification of fruit or plant characteristics that cause the drying process to start would be useful to aid in the selection of seedlings for natural DOV raisins. The drying rates at 38C of two natural DOV selections were compared to Summer Muscat (fast berry drying rate) and Thompson Seedless (slow drying rate). All berries compared were 14.3 mm diameter and 22^{o} Brix determined by density flotation. The drying rate of one genotype was similar to Summer Muscat and the other dried slower than Thompson Seedless, even though both dry into raisins on the vine without cutting canes. Drying was slower for all genotypes when the pedicle was covered with wax to prevent water loss through the pedicle compared to two years when the pedicle was not waxed. Thompson Seedless drying rate was dramatically decreased by the wax compared to the other genotypes. The drying order was the same for all years except Thompson Seedless was the slowest when waxed. The drying rate does not explain why the two natural DOV genotypes dry without cutting canes. The cuticle, wax and skin thickness is being compared to see if they might be influencing drying rate. The DOV genotypes do not develop high sugar levels early as might be expected. There may be other factors such as water transport out of the berry and through the leaves that might be causing the initiation of the natural DOV phenomena.

P-92

Quality improvement in Vignoles through clonal selection

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Vignoles is a valuable component of the variety mix in the eastern United States. Consumers appreciate its distinctive apricot, peach, and citrus notes in wines. Because it is relatively winter hardy (reliably to – 26 ºC), Vignoles is grown in such diverse locations as New York, Missouri, Indiana, Ohio, Pennsylvania, Illinois, Nebraska and Michigan. However, Vignoles has small, tight clusters prone to bunch rot later in the season, and losses up to one third of the crop are possible in rainy years. Despite consumer demand for Vignoles, the production of these wines is limited by bunch rot susceptibility associated with compact cluster architecture. Development of chemical and cultural practices to counter bunch rots in Vignoles remains challenging. Our goal is to select an improved, loose-clustered clone of Vignoles that will contribute to an integrated approach to disease control. Following gamma radiation, irradiated buds and non-irradiated controls were bench grafted onto 3309C rootstock. A total of 2336 vines, including 30 non-mutagenized Vignoles control vines, were planted in 2007 (500 vines) and in 2008 (1839 vines). The experimental vineyard is located at the Cornell Lake Erie Research and Extension Laboratory (Portland, New York) in the Lake Erie shore grape and wine production region. The clones were planted at a spacing of 4 feet between vines and 9 feet between rows. Vines were cultivated in grow tubes and drip irrigated to promote rapid vine establishment and growth. The vines were trained to a bilateral cordon, spur pruned, and cultivated following standard practices for hybrid wine grape varieties in western New York, including fertilization and weed, pest, and disease management. Many clones planted in 2007 fruited in 2008, but fruit was dropped to encourage vegetative establishment. The generation of new clones and the establishment of the vineyard are complete. The second stage of the project is evaluation of cluster looseness and bunch rot susceptibility. A pyramidal selection approach for cluster compactness is being followed, using berries per centimeter as a measure of cluster compactness. Screening in the population of clones has identified several clones with looser clusters than control, non-mutagenized Vignoles vines.

P-93

Development of table and raisin grapes with high anthocyanins using a leaf disk assay

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Anthocyanins are considered an excellent source of antioxidant phytochemicals for health benefits. The majority of wine, table and raisin grapes have anthocyanins only in their colored skin. Anthocyanin content of grapes would be increased if their flesh also contained anthocyanins. The 'Rubired' wine grape has black skin and red fleshed berries. It was used as a pollen parent to introgress red flesh in a cross with a seedless female flowered table grape selection C33-30. It has red skin and white flesh berries. The F1 progeny segregated in a 3 colored:1 white skin and 1 red:1 clear flesh ratio for colored berries. Seedlings expressing the highest level of anthocyanins in the flesh and skin were equivalent to 'Rubired'. Seven modified backcross families were created by crossing red flesh seeded F1 selections with seedless red skin table or white skin raisin grapes. Skin color segregated as a single dominant gene as expected. One red flesh parent was heterozygous for skin color and the rest were homozygous. Only seedlings with colored skin had red flesh. Flesh color segregated as a 1 red:1 clear in three of the families or in a 2:1 ratio indicting either a single dominant gene or several genes, with red flesh being predominant. One red flesh parent produced an abnormally high percentage (>80%) of red flesh seedlings. The assay consisted of placing leaf disks in 10% sucrose solution and observing the development of anthocyanins after 5-10 days under 12 hr photoperiod at 26C. When no anthocyanin developed in the leaf disks, their seedlings had clear flesh with white, red or black skin (30% of population) and could be discarded with confidence. Red skin seedlings with light red to red flesh as well as black skin with dark red flesh were identified accurately (>50% red in leaf disk). In some cases seedlings with red or black skin and clear flesh produced anthocyanins in the leaf disks and were not easily differentiated from black skin with light red or red flesh.

P-94

Mechanisms of powdery mildew resistance in the Vitaceae family

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The cultivated grapevine*, Vitis vinifera*, is a member of the Vitaceae family which comprises over 700 species in 14 genera. *Vitis vinifera* is highly susceptible to the powdery mildew pathogen *Erysiphe necator.* However, other species within the Vitaceae family have been reported to show resistance to this fungal pathogen but little is known about the mechanistic basis of resistance in these species. Plants secrete cell wall materials at the site of attempted fungal infection in order to block penetration. If this penetration resistance is overcome some plant species have evolved resistance genes, the products of which trigger programmed cell death (PCD) in the presence of the host fungus. Therefore, the frequency of successful *E. necator* penetration events, in addition to PCD responses, were investigated in a range of different species within the Vitaceae family. The results reveal that penetration resistance and PCD-mediated responses or combinations of both are employed by species from the different Vitaceae genera to limit *E. necator* infection. In order to further characterise the cellular processes involved in the observed penetration resistance, specific inhibitors of the actin cytoskeleton and secretory/endocytic vesicle trafficking function were employed. These inhibitors were demonstrated to successfully break penetration resistance in *V. vinifera* against the non-host powdery mildew *E. cichoracearum*. However, the use of these inhibitors with the host powdery mildew *E. necator* unexpectedly revealed that while secretory and endocytic vesicle trafficking pathways play a crucial role in non-host penetration resistance, the host powdery mildew species may actually require these pathways to successfully penetrate the plant host.

P-95

Reaction of grape rootstocks to *Meloidogyne incognita* and *M. javanica*

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This study evaluates, under controlled conditions, the reaction of ten grape rootstocks from a germplasm collection from the Programa de Melhoramento Genético de Videira at the Centro APTA de Frutas do Instituto Agronômico de Campinas, Brazil, to *Meloidogyne incognita* and *M. javanica* nematodes. Seedlings were inoculated with 4,000 eggs and juveniles from these nematodes. After 60 days, radicular systems were evaluated with respect to the amount of galls in order to determine, through calculation of the reproduction factor, the immunity, resistance and susceptibility of rootstocks. Rootstocks '101-14', 'Teleki-5C', 'IAC 313', 'IAC 766', 'Kober 5BB' and 'Ripária do Traviú' behaved as immune to both species of nematodes – 'R99' presented resistance. 'IAC 571-6' and 'IAC 572' behaved as resistant to *M. incognita* and immune to *M. javanica*; '420-A' was immune to *M. incognita* and resistant to *M. javanica*.

P-96

Global gene response to viral infections in grapevine

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Viral infections in grapevine develop compatible interactions, in which pathogens spread through all plant tissues without inducing a resistance response but affecting morphology of leaves, vegetative growth and productivity. Also, organoleptic properties, acidity and sugar content of the fruit are affected by the viral infections which affect the quality of the must produced from these infected plants. To investigate the global effect of virus infections in grapevine plants, transcriptional profiles of the red wine cultivar 'Cabernet Sauvignon,' which was naturally infected with GLRaV-3, were compared with virus-free grapevine plants. The Affymetrix GeneChip® Vitis genome array, version 1.0 was using for the analysis of leaves and berries at two maturation stages: veraison (EL-35) and maturation (EL-38). The infection was confirmed by RT-PCR and also by electronic microscopy that allowed us to observe virus particles in leaves and berries of the infected plants. A wide spectrum of biological functions was affected by viral infections in all tissues studied. The most relevant changes in gene expression in leaves were associated to synthesis and destination of proteins, transport and metabolism. Also developmental processes, senescence and cell defence were affected. In the fruit, the virus infection affects the maturation process, since a lower number of genes change expression at veraison and maturation stages in the infected plants compared with healthy plants. Besides, an important number of genes that appear down regulated in the infected fruits at maturation stage are associated with processes of biosynthesis of primary and secondary metabolites, metabolism, development process, senescence and cell defence. Therefore the maturation process appears incomplete in the infected plants.

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Genetic analysis of the resistance to foliar diseases on *Muscadinia rotundifolia*

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Viticulture is an important component of French agriculture, both in economical and cultural terms. Neverthless, a wide range of pathogens threatens French viticulture. The current strategy to control most grapevine diseases totally relies on the use of fungicides. This practice is not only expensive but also dangerous for the environment and human health.
A cost-effective and environment friendly alternative to chemicals is the use of resistant varieties. However, all cultivated European grapevine varieties are susceptible to most of the pathogens, thus the resistance needs to be introduced from other *Vitaceae* through breeding programs. *Muscadinia rotundifolia* (2n=40) is a species closely related to the true *Vitis* (2n=38) and shows a high level of resistance to a wide spectrum of grapevine diseases.
In order to analyse and exploit the syntheny between *V. vinifera* and *M. rotundifolia* and to accelerate the functional characterization of resistance to pathogens, we are developing a genetic map of *M. rotundifolia*, with a set of SSR markers scored on a 200 individuals S1 population of *V. rotundifolia* cv Regale. 150 markers out of 350 SSR have been found heterozygous, thus informative for mapping. Construction of the map is in progress.
In parallel, the same population was tested for its resistance to downy mildew (Plasmopara *viticola*). Macroscopic and cytological observations on leaf discs allowed us to assess the level of resistance of these genotypes to downy mildew. The whole progeny is very resistant to *P. viticola.* Stomatic necrosis were frequently observed that may lead, in most cases, to stop the invasion of the pathogen when the biotrophic step of its life cycle starts, and then to inhibit sporulation. In few genotypes, downy mildew penetrate the leaf and develop haustoria and mycelium. In this case, a weak sporulation occurs very rarely. Our next goal will to align *M. rotundifolia* and *V. vinifera* genetic maps, in order to understand their degree of syntheny and to locate the position of the clusters of resistance genes in *V. rotundifolia*. The further analysis will help to understand the resistance mecanism of *V. rotundifolia* to pathogens.

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Investigation of the interaction of *Elsinoë ampelina* with *Vitis vinifera*

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Grapevine anthracnose is a disease of European origin that reduces the quality and the quantity of the crop. The causal fungus is *Elsinoë ampelina* (de Bary) Shear. Previously, we sought the optimum conditions for a simple method to stably generate conidia, and found a high negative correlation between the colony density on potato dextrose agar (PDA) medium and the number of resultant conidia. (Kono et al., 2009). The conidia produced by this method were pathogenic to susceptible *Vitis vinifera.* Two days after the inoculation of the conidial suspension, small collapse on the surface of the leaves were observed. The color of the collapsed area was almost green, which is similar to that of the healthy part of the leaves. Visible brownish lesions were formed after four to five days post-inoculation. However, the green collapsed cells were observed in the peripheral area of the brownish lesions even after seven days post-inoculation, suggesting that the fungus actively invaded in the healthy cells without inducing any defense responses. One of the important processes of defense responses is the hypersensitive response (HR), which is a localized self-induced cell death at the site of infection. HR inhibits pathogen growth by the release of toxic compounds, and also leads to the activation of numerous other defense-related genes. We used trypan blue staining in order to stain both the fungal cells and the site of the HR. After the staining, deeply stained cells of the grapevine were observed in the central part of the lesions. However, bluish hyphae were observed in the peripheral area of the lesions surrounded by the leaf cells that were not stained with trypan blue, which suggested that the surrounding cells of the hyphae did not show the HR. These observations showed that the mycelia of *E. ampelina* actively invaded into the living *V. vinifera* cells without inducing the HR, suggesting that the absence of the HR in *V. vinifera* might be the cause of the susceptibility to *E. ampelina.*

P-99

Berry cracking caused powdery mildew (*Uncinula necator*) infection in 'Fujiminori' grapevines (*Vitis* sp.)

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Powdery mildew (*Uncinula necator*) is one of the common grape diseases in protected rain-shelter cultivation in Korea. Wounds from disease infection may provide the starting point of cracking in susceptible tetraploid grapevines. To find out the effect of *U. necator* infection on berry cracking, morphological and histological observation was conducted using the cracking susceptible 'Fujiminori' grape cultivar. The intact berry rate was lower after the inoculation (85.8%) than in the control vines (89.3%). Cluster weight and numbers of berries per cluster in inoculated vines was significantly lower, with 722.6 g and 38.6 compared with 842.2 g and 40.2 in controls. Inoculation with powdery mildew reduced the firmness of pericarp to 1.11 kg/Ø5mm from the 1.31 kg/Ø5mm of controls. The berry cracking rate under critical turgor pressure was also decreased from 18.0% to 29.0% after inoculation of *U. necator*. The degree of cell wall degradation was measured by cell wall content from 10 g pericarp. The cell wall content of inoculated 'Fujiminori' was reduced 31.3% with collection of 311.7 mg compared with untreated vines of 453.7 mg. Polygalacturonase, one of the major enzymes inducing cell wall degradation, activity was increased from 0.143 to 0.204 units/mL in *U. necator* infected berries. In the results from powdery mildew inoculation, the invasion of hypha in early berry growth stages before veraison promotes cell breakdown between the sub-epidermal and the epidermal layer by secreted cell wall degradation enzymes even no visible disease symptom in the berry.

P-100

Gray mold (*Botrytis cinerea*) induced berry cracking in 'Fujiminori' table grapes (*Vitis* sp.)

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Tetraploid grape cultivars including 'Fujiminori' are cultivated for higher quality and marketability in table grape production using protected rain-shelter cultivation system in Korea. Gray mold (*Botrytis cinerea*) is one of the most serious diseases for tetraploid grape cultivation. To find out the berry cracking symptoms caused by *B. cinerea* infection, morphological and cellular characteristics were observed using cracking susceptible 'Fujiminori' grapevines. The intact berry rate indicative of marketability was decreased by 62.7% after the inoculation compared to 89.3% in the control vines. Cluster weight and numbers of berries per cluster in inoculated vines was also significantly decreased as 535.6 g and 28.2 compared with 842.2 g and 40.2 in non-inoculated controls. The decreasing of berry firmness from 1.31 to 1.08 kg/Ø5mm in gray mold inoculated vines induced a three-fold higher berry cracking rate under critical turgor pressure from 18.0 to 52.0%. The cell wall content per 10 g of pericarp, which can be an indicator for strength of cell wall structure, was reduced 45.9% from 453.7 to 245.3 mg in gray mold infected 'Fujiminori' vines. The increase in polygalacturonase activity from 0.143 to 0.204 units/mL after the infection of *B. cinerea* could also accelerate the cell wall degradation and berry cracking. Though there are no visible disease symptoms on the berry surface in early growth stages before veraison, invaded hypha caused by *B. cinerea* infection are already promoting cell breakdown between the sub-epidermal and the epidermal layer by secreted cell wall degradation enzymes. Therefore, the susceptibility and the infection of *B. cinerea* before veraison can be an important trigger of berry cracking.

P-101

Elicitation of grapevine defense responses against downy mildew caused by *Plasmopara viticola*

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Plasmopara viticola, the casual agent of downy mildew, is one of the most destructive grapevine diseases in Europe and in the eastern half of the United States. In recent decades, induced resistance to *P. viticola* has gained increasing attention as a strategy to control the disease in a more sustainable way. This work aims at triggering the plant's own defence mechanism/s against *P. viticola* by some selected elicitors, through prophylactic as well as curative strategies. To evaluate the level of induced resistance, an integrated approach will be followed to assess the efficacy of the elicitors used as well as to understand their mode of action. This includes synthesis of phytoalexins as well as PR-proteins, cell death (hypersensitive response) and defense gene and protein expression, to name a few. Disease severity as well as disease development have been assessed. Callose formation was measured as an indicator of the activation of a defence reaction. First experiments indicated different infection intensities as a result of different elicitor applications, which in turn indicateed that some elicitors had the effect of reducing the spread of the disease. For disease development, haustoria formation and hypersensitive response were measured using epifluorescence microscopy. At the molecular level, the gene expression of some candidate genes was measured using real-time PCR. The initial results showed differential expression of these genes between the different treatments.

P-102

Optimizing the breeding of Pierce's disease resistant wine grapes with marker-assisted selection

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Pierce's disease (PD) greatly limits the production of *Vitis vinifera* based grapevines across the southern United States. The disease is caused by a xylem-limited bacterium, *Xylella fastidiosa*, and is spread by the sharpshooter family of leafhoppers (Cicadellide). Many *Vitis* species in the southern United States are resistant to *X. fastidiosa*, however their resistance is multi-genic and complex, which has limited breeding progress. However, resistance from several forms of *V. arizonica* (b43-17 and b40-14) from northern Mexico is inherited as a single dominant gene; all F1 generation offspring are resistant and F2 populations segregate 1:1 resistant:susceptible. This resistance has been genetically mapped to chromosome 14 where the locus, *PdR1*, is flanked by two closely linked SSR markers. Physical mapping identified a 201Kb region that encompasses *PdR1*, which is currently under study. The tightly linked flanking markers have been used for marker-assisted selection (MAS) for *PdR1*. MAS in conjunction with aggressive training in the field has brought seed-to-seed cycle down to two years. In 2010, a total of 4,360 seedlings from 34 crosses of modified BC4 seedling populations (97% V. *vinifera*) were marker tested and over 2,000 resistant seedlings were planted in the field. These are destined for fruit and wine evaluations in 2012 prior to commercialization tests. Small-scale wines have been made from BC3 94% *V. vinifera* PD resistant selections with very promising results. PD resistant selections from the BC1, BC2 and BC3 level have also been planted in a severely infected area in Napa Valley. These plants are hand inoculated each year and their symptoms and bacterial titers are evaluated and compared to known resistant and susceptible controls.

P-103

The biological characteristics of *Xylella fastidiosa* in xylem fluid from resistant and susceptible grapevines

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Xylella fastidiosa (*Xf*) is a fastidious and xylem-limited Gram-negative bacterium that causes Pierce's disease (PD) on grapevine. It was investigated that while grapevine *V. vinifera* is susceptible to the PD strain of *Xf*, *V. champinii* and *V. smalliana* are tolerant or resistant to the PD strain. The biological characteristics of *Xf* PD strain were investigated by examining the *in vitro* effect of pure xylem fluid from resistant and susceptible grapevines on *Xf* multiplication, aggregation, and attachment. The aggregations of large clumps were formed in pure xylem fluid from susceptible grapevine, whereas small clumps were observed in resistance grapevines xylem fluid. Microarrays are being applied for analysis of differential gene expression files of *Xf* PD strain in xylem fluids from different grapevines. Xylem fluid is being analyzed to determine the chemical compounds or elements that control the virulence of *Xf*. The motility of *Xf* in differential xylem fluids of grapevines is being observed within micro-channels.

P-104

Development of marker-assisted selection as a tool for breeding disease resistant grapes

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The Cornell grape breeding program has a long-standing effort to develop disease resistant grape cultivars. A number of molecular markers that are closely associated with disease resistance loci have been identified. We sought to examine a range of molecular markers for resistance loci and determine their utility in populations already in use in the grape breeding program. Populations of seedlings were screened in the laboratory for downy mildew resistance using detached leaves. In a field nursery, populations were screened for resistance to powdery mildew. Field grown seedlings potentially carrying both the *Rpv1* and *Run1* loci for downy and powdery mildew resistance, respectively, from muscadine (*Vitis rotundifolia*) introgressions were also assessed for resistance to both mildews. In these populations, markers for the *Run1* gene were excellent predictors of powdery mildew resistance, but markers for the *Rpv1* gene for downy mildew resistance from the muscadine grape were not useful in predicting downy mildew resistance. Markers for the *V. cinerea* source of powdery mildew resistance were also found to be useful. Populations that were previously screened for powdery mildew resistance (where the susceptible types had already been discarded) had a very high frequency of markers for the *Run1* gene and/or the *V. cinerea* source of resistance, confirming the success of field screening techniques. By screening for presence of markers, it was possible to identify seedlings harboring both the *V. cinerea* as well as muscadine sources of powdery mildew resistance.

P-105

Pathogen responsiveness and variety/tissue specificity of five stilbene synthase genes in grapevine

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Grapevine stilbenic compounds have antimicrobial activities and some of them including resveratrol are beneficial to human health. Stilbene synthase (STS) is the key enzyme that catalyzes the biosynthesis of stilbenic compounds. Grapevine genome contains 43 STS genes, and the regulation of each STS gene needs to be understood for defining their roles. In this study, we selected five STS genes and employed quantitative polymerase chain reaction (qPCR) to characterize their transcriptional profiles during the berry development and infection by the powdery mildew fungus (PM), *Erysiphe necator* (Schw.) Burr., in *Vitis vinifera* 'Cabernet Sauvignon' and *Vitis aestivalis* 'Norton'. We found that transcript levels of two STS genes increased along the berry development and were significantly higher in Cabernet Sauvignon than in Norton. Transcript levels of other three STS genes increased during the berry ripening, and were higher in Norton than in Cabernet Sauvignon. In response to PM infection, four STS genes were up-regulated in Cabernet Sauvignon leaves while three STS genes were induced in the Norton leaves. These results demonstrated that each STS gene plays a special role in the berry development and in the defense against fungal infection, and revealed new insights into the regulation of the STS gene family in grapevine.

P-106

Characterization of grapevine NPR1

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Non-expressor of Pathogenesis-Related genes 1 (NPR1) is a key transcription factor in the defense against pathogens by regulating defense-related genes in plants. Mutation of the *NPR1-1* gene in Arabidopsis plants reduces transcript levels of pathogenesis-related (PR) genes including PR-1 and makes *npr1-1* mutant more susceptible to pathogens. There are two NPR1 genes in the grapevine genome. It is known that these two grapevine NPR1 genes are activated by salicylic acid analog, but their functions are still based on the bioinformatics prediction. In the present study, one full-length NPR1 gene was cloned and sequenced from *Vitis aestivalis* 'Norton' and *Vitis vinifera* 'Cabernet Sauvignon', and referred to as *VaNPR1.1* and *VvNPR1.1*, respectively, based on the high identity to the previously designated *NPR1.1* of grapevine. Bioinformatics analysis indicated that predicted amino acid sequences of VaNPR1.1 and VvNPR1.1 differ by one amino acid. Over-expression of *VaNPR1.1* gene in *Arabidopsis npr1-1* mutant plants restores the expression of PR-1 gene, though not to the full scale. This result demonstrated that VaNPR1.1possesses similar function to *Arabidopsis* NPR1 in the regulation of defense-related genes and thus verified the function of grapevine NPR1.1 gene.

P-107

Screening grape hybrid families with molecular markers linked to resistance genes

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Gene pyramiding is a method to breed grape varieties for durable disease resistance. The use of resistance gene-linked DNA markers reduces the volume of breeding experiments, since it facilitates the identification of progeny seedlings that have inherited the desired gene shortly after germination and allows the reduction of population size. Saturating the grape genome with molecular markers, the construction of genetic linkage maps, and the recent publication of the *Vitis vinifera* genome sequence enable breeders to select the desired genotype directly. To combine powdery and downy mildew resistance genes, Kozma *et al*. (Research Institute of Viticulture and Enology, Pécs) produced the following hybrid families: BC_4 (VRH 3082-1-42) x 'Kishmish vatkana', BC_4 x 'Kishmish moldavskij', 'Génuai zamatos' x 'Kishmish vatkana', ('Laszta' x. 'Dzhandzhal kara') x ('Katta kurgán x Perlette'), where BC_4 derives from a *V. vinifera* x *Muscadinia rotundifolia* cross. Our aim was to select the individuals containing the powdery (PM) and/or downy mildew (DM) major resistance genes of different origin (*Muscadinia rotundifolia-Run1, Rpv1, V. vinifera-Ren1,* and PM and DM QTLs of 'Seyve-Villard') with SSR, CB and SCAR markers. PCR products were separated on 8% polyacrilamide (ALFExpress) and 4% Metaphor gel. Our data corroborated earlier findings that the *M. rotundifolia*-derived *Rpv1* and *Run1* loci are closely linked. We compared the symptomless progenies derived from the cross BC_4 x 'Kishmish vatkana' and ('Laszt' x 'Dzsandzsal kara') x ('Katta kurgán' x 'Perlette') using resistance linked markers. We observed that alleles of SSR markers linked to the PM resistance gene *Ren1* in the linkage group 13 are the same, suggesting that PM resistance locus of 'Kishmish vatkana' and 'Dzhandzhal kara' are presumably identical. Studies of QTL markers are in progress.

P-108

The genetic mapping of *Xiphinema index* resistance derived from *Vitis arizonica* by using SSR markers

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A breeding program to develop rootstocks with resistance to *Xiphinema index,* the dagger nematode vector of grapevine fanleaf virus (GFLV), has been underway for many years at UC Davis. Strong resistance to *X. index* was discovered in a hybrid form of *Vitis arizonica/girdiana* (b42-26). Inheritance studies found its resistance to be controlled by a single dominant locus, which was later genetically mapped and named *XiR1*. In this study we are examining *X. index* resistance derived from a pure form of *V. arizonica* b40-14 that was collected from Chihuahua, Mexico. A mapping population (0705) was derived from the susceptible rootstock 161-49C crossed with 8916-22 (*V. rupestris* A. de Serres x b40-14), which is heterozygous for *X. index* resistance. Preliminary greenhouse screening results suggest a 1:1 resistant to susceptible segregation ratio for *X. index* in this population. To develop a genetic map, over 400 SSR markers were tested on a small set of genotypes that included the parents and a few progeny. A total of 190 markers were polymorphic for either or both parents, covering all 19 linkage groups. One hundred and forty of the polymorphic markers have been completed on the entire set of 187 progeny in the 0705 population. The addition of more SSR markers to saturate the linkage groups is underway. A moderately saturated genetic map will be used to identify the genomic regions and markers associated with *X. index* resistance that could be used for marker-assisted breeding programs and as a foundation for fine-scale mapping.

P-109

Characterization of an ankyrin-like protein up-stream region from *Vitis vinifera* is highly induced by *Botrytis cinerea* infection

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Grey mold, caused by *Botrytis cinerea*, is a main grapevine disease in Chile. *B. cinerea* is a necrotrophic filamentous fungus of a complex agricultural management because of a broad host spectrum and to the different sources of inoculation. In the "Genome I: Botrytis-Grape Interaction" project, a macroarray formed by 4803 ESTs was designed. Gene expression studies using this macroarray allowed the comparison between mRNAs from infected and non-infected grapevine field plants from two contrasting cultivars: *Thompson Seedless* and Carménère. By this analysis, we established the involvement of a putative ankyrin-like protein highly induced in response to the pathogen infection. Although is not expected to find natural resistance sources to *Botrytis* in breeding grapevine studies using susceptible cultivars, the knowledge of highly responsible sequences to fungal challenge could be of significant relevance from a biotechnological approach. In this way, we propose that the promoter region of this gene may contain important responsive element which may provide information about the specificity of the plant response after fungal attack. A first step in this work was to verify and compare the observed induction of the grapevine ankyrin-like gene under fungal attack by specific *real time* PCR analysis of infected Carménère greenhouse plants. Afterwards, *in silico* analysis was carried out leading to the definition of an 890 base pair up-stream zone, which was experimentally characterized by use of GFP fusion constructs in tobacco transformation experiments. Wounding and Botrytis challenges were applied on stable transgenic lines, depicting differentially induced levels of *gfp* transcripts depending on the elicitor. Comparison of these heterologous inductions is under development by generation of the corresponding ´Thompson Seedless´ transgenic lines.

P-111

Cloning and functional characterisation of a putative powdery mildew susceptibility gene in grapevine

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Grapevine is highly susceptible to a range of different fungal pathogens including powdery mildew (*Erysiphe necator*), which can cause severe reductions in yield and quality. As part of multifaceted approach to improve the genetic resistance of grapevine to powdery mildew, we are investigating the grapevine pectate lyase-like (PLL) gene family because previous research in Arabidopsis has demonstrated that a specific member of the *AtPLL* gene family (*PMR6*) was required for powdery mildew susceptibility. Sixteen *VvPLL*s were predicted to be present in the grapevine genome. Based on sequence homology, we identified three genes, designated *VvPLL1*, *VvPLL2* and *VvPLL3*, as potential orthologs of *PMR6*, and the coding regions were amplified from *Vitis vinifera* 'Cabernet sauvignon'. All three *VvPLL* candidates were found to encode proteins with a C-terminal GPI-anchor, which is a characteristic of *PMR6 and Vv*PLL-GFP fusion constructs confirmed the VvPLL proteins were targeted to the plasma membrane. Quantitative RT-PCR analysis indicated constitutive expression of *VvPLL1*, *VvPLL2* and *VvPLL3* in all tissues examined including leaves, roots, flowers and berries. In contrast, other members of the *VvPLL* gene family such as *VvPLL9* and *VvPLL10* were tissue-specific (floral). In agreement with observations for *PMR6*, *VvPLL1*, *VvPLL2* and *VvPLL3* were not significantly up-regulated by powdery mildew infection. Functional complementation experiments are currently underway in which the three *VvPLL* genes have been transformed into the *Atpmr6* mutant under the control of either the CaMV 35S or Arabidopsis *PMR6* promoter. Our ultimate aim is to silence these *VvPLL* genes in grapevine to determine if we can modify susceptibility to powdery mildew.

P-112

Evaluation of grapevine germplasm from La Rioja for tolerance to powdery mildew (*Erisiphe necator*)

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Powdery mildew is caused by the plant pathogenic fungus *Erisiphe necator* and is one of the most important and destructive diseases of grapevine in many countries of the world, including Spain. Development and use of tolerant varieties is one of the most important and environmentally sound strategies for management of this disease. Susceptibility or tolerance to powdery mildew infection were evaluated on shoots in 49 accessions of the germplasm collection from an experimental field at La Rioja University (Spain). The natural infections were scored during 2008-2009 using a six point scale. No pesticides were used in the experimental sites, and a high severity of infection was observed, with all accessions infected by the fungus but showing differences in the severity levels. The more tolerant were R-14, SO-63, CI-78 and the more susceptible accessions were H-29, AR-43, B-47 and AB-100. The results were promising as they may be used as a tolerant genetic sources for breeding to manage the disease.

P-113

Analysis of genetic structure of twelve Sicilian grapevine cultivars

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In recent years, Sicilian viticulture has shown a significant growth with a remarkable increase of production, both in quantity and in quality. Sicily is a wide wine region in Italy and its wines have already made themselves known on the international markets. Several autochthonous grapevine cultivars have been preserved in Sicily, but this available resource is mainly unexploited because of the lack of wide genetic characterizations. The aim of the present study is to investigate molecular characteristics of Sicilian cultivars; evaluate probable cases of synonymies and false attributions and preserve Sicilian germplasm. A wide screening on 12 major Sicilian cultivars was carried out (*Carricante, Catarratto comune e lucido, Nero d'Avola, Nerello cappuccio, Nerello Mascalese, Perricone, Grillo, Grecanico, Inzolia, Corinto nero* and *Frappato*). A total of 687 accessions were sampled by the use of SSR markers (until 21 *loci*). Allelic profiles were obtained and confronted with other data banks, allowing to exclude some false attributions. Molecular analysis clarified several synonymies and false attributions: in particular there are many cases of *Sangiovese* cultivated as *Nerello mascalese* and *Corinto nero;* furthermore this comparison highlights that the 70% of *Nerello cappuccio* accessions is actually *Carignan.* A total of 25 unique and different SSRs profiles were found. Several cultivars, such as *Perricone, Carricante* and *Grecanico,* showed a high level of genotypic variability with different allelic profiles. No differences were detected among the accessions of *Catarratto comune* and *Catarratto lucido*; these cultivars have the same allelic profile. Parentage analysis suggested origins of some cultivars: *Nero d'Avola, Grecanico, Catarratto* and *Inzolia* are autochthonous while *Nerello cappucio*'s profiles can be closely related to some Calabrian cultivars. These results highlight the genetic richness and variability that is still present in the Sicilian region and that can be probably explained by its geographical isolation; furthermore, they provide a solid base for future studies and developments.

P-114

Evaluation of four new rootstock genotypes obtained by backcross

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In viticulture, the introduction of rootstocks was tied to the appearance of phylloxera in Europe in the late 1800s; today their use is strongly related to grapes adaptation to different pedo-climatic environments. If the extreme variability that characterizes agricultural habitats is taken to account, some specific and complex needs come out, such as the adaptation of rootstocks-cultivar pairs to soil features and the possible use of rootstocks for modulating the grapevine's agronomical responses to edaphic and meteorological conditions. Starting from these assumptions, the introduction of new rootstocks can be a valuable contribution to the optimization of vineyard vegetative and productive responses. With this aim, the present research was started to compare four new rootstocks, obtained by Di. Pro. Ve. (University of Milan) through a back cross breeding selection, to six commercial ones (41B, 420A, 110R, 140Ru, 1103P and SO4). Four experimental vineyards (respectively placed in the "Valpolicella", "Chianti Classico", "Castel del Monte" and "Contea di Sclafani" A.O.C. areas) were planted with these ten rootstocks grafted to 'Cabernet Sauvignon' and a local variety. In those vineyards, 50 plants of each parcel (combinations of roostock-cultivar) were placed in randomized blocks, at about 5000 plants/ha and trained as a spur pruned cordon. From 2007 to 2009 and on a representative number of plants for each parcel some observations were made during vintage and winter pruning: agronomical parameters (Ravaz index, bud fertility, production weight/plant, bunches average weight, wood weight) and productive qualitative ones (pH, titratable acidity, soluble solids of musts, polyphenols and anthocyanins total contents of berry skins) were collected. Where it was possible, grapes were made into wines using a standard microvinification protocol, and wines were chemically analyzed and sensory evaluations were carried out. Our results showed that the new genotypes cause improved qualitative performances; have different capabilities to modulate cultivars' vigor; induce high accumulations of soluble sugars in musts and good quantities of production/plant; and finally, are highly resistant to water stress. In conclusion, these new rootstocks could be introduced into commercial settings and could allow to better modulation of viticultural production in different pedo-climatic environments.

P-115

Genetic transformation of grape (*Vitis vinifera*) to increase salt tolerance

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Chile is one of the main table-grape producer and exporter worldwide. However, in Northern regions the crop expansion is limited because of environmental conditions and desertification process affecting soil quality and plant productivity. Salinity is a major constrain, with soil conductivities over 4 dS/m. Under these conditions, grape yield is reduced more than 10%, severely affecting productivity. Among the strategies to improve abiotic stress tolerance in crops, genetic transformation with *Arabidopsis* transcription factors CBF/DREB1 has been successfully employed, resulting in plants with increased drought, salt and/or cold tolerance. Additionally, some undesirable dwarf phenotypes had been reported, because up-regulated CBF/DREB1 expression. In order to generate salt tolerant grapes, binary vectors expressing the Arabidopsis CBF3/DREB1A gene (AtCBF3) driven by either the constitutive CaMV 35S or abiotic stress inducible rd29A promoter were engineered for transformation of *Vitis vinifera* cv. Thompson seedless plants. Transformant lines were confirmed by PCR and were transferred into standardized hydroponic cultures and further salt treatments were applied. Basal culture medium (Phostrogen, NPK 14:10:27 plus trace elements) was supplemented with NaCl 100 mM and plants were grown for 35 days to evaluate salinity tolerance. Photochemical efficiency (Fv/Fm) and total chlorophyll content were measured once a week either in transformed and non-transformed plants (wild type controls). We detected that eight transgenic lines (four containing prd29A:AtCBF3 construct and four containing p35S:AtCBF3) displayed better growth, photochemical efficiency and chlorophyll content under these assay conditions. No pleiotropic phenotypes were observed due to constitutive expression of AtCBF3. Additional determinations are being conducted in order to characterize these tolerant grapevine lines and the transgene transcriptional dynamics under experimental conditions.