

**EUCARPIA FRUIT SECTION**

**THIRTEENTH EUCARPIA SYMPOSIUM ON FRUIT  
BREEDING AND GENETICS**

**Warsaw, Poland, September 11-15, 2011**

**Department of Pomology, Warsaw University of Life Sciences (WULS – SGGW)  
and the Committee of Horticultural Sciences of the Polish Academy of Sciences**

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## FOREWORD

It is a great pleasure to welcome all of you, interested in fruit breeding and genetics, at the XIII Eucarpia Symposium in Warsaw. We are happy to have an opportunity to host the specialists from all over the world!

Conventional breeding of new fruit cultivars faces many limitations, like a very long juvenile period of seedlings, large extension of land to grow them and a large size of a progeny to be screened. New cultivar ought to satisfy expectations not only consumer, but also fruit growers, commercial and industrial sectors. Modern molecular techniques enable to overcome these limitations. We meet together to share knowledge and experiences and exchange ideas. This should help to develop new, more efficient breeding programmes and open new perspectives. I wish fruitful debates at the sessions and inspiring discussions in lobbies as well! On this occasion I take also the liberty to thank to all the members of the Organizing, as well as the Scientific and Editorial Committee for their large-hearted co-operation in organizing the Symposium.

Finally I wish you meeting old friends, establishing new friendships and an overall pleasant stay in our city!

Emilian Pitera  
Symposium Convener

The Organising Committee gratefully acknowledge the financial support provided by the following organisations for the Symposium



# Programme of the Symposium

## REGISTRATION, ORAL SESSIONS, AND FIELD TRIP

### Sunday 11 September

17:15	Departure from the Sofitel Hotel
17:30	Departure from the Ibis Hotel
18:00 - 19:00	Registration
19:00 - 21:00	Welcoming reception
21:00	Departure to hotels

### Monday 12 September

07:30	Departure from the Ibis Hotel
08:15 - 09:00	Registration
09:00 - 09:30	Opening ceremony <b>Prof. dr Kazimierz Banasik</b> , Rector's Attorney for International Cooperation of Warsaw University of Life Sciences – SGGW <b>Prof. dr Marek S. Szyndel</b> , Dean of Faculty of Horticulture and Landscape Architecture of Warsaw University of Life Sciences – SGGW <b>Prof. dr Silviero Sansavini</b> , Representative of the ISHS, Past President <b>Dr Emilian Pitera</b> , Chairman of the Symposium
09:30 - 09:50	Fruit trees breeding in A.A. Przybyła Poland.

### Oral session: Breeding programmes and new cultivar development

**Chairs: A. Sadowski and K.M. Evans**

09:50 - 10:20	Apple breeding and genetic control of the main agronomic, resistance and fruit quality traits. (invited lecture)	S. Sansavini* and S. Tartarini	page 29
10:20 - 10:40	40 years of Australian National Apple Breeding Program.	F. Shan	page 30
10:40 - 11:00	A new almond breeding program in Turkey.	I. Acar*, S. Arpacı, H.S. Atlı, S. Kafkas, S. Eti, S. Caglar, and A. Yilmaz	page 31
11:00 - 11:20	Apple breeding and genetic resources in Latvia.	L. Ikase* and G. Lacis	page 32
11:20 - 11:50	<b>Break</b>		

**Oral session: Breeding programmes and new cultivar development**

**Chairs: S. Sansavini and E. Żurawicz**

- |               |  |   |         |
|---------------|--|---|---------|
| 11:50 - 12:20 | Apple breeding in the Pacific Northwest. (invited lecture)   | K.M. Evans  | page 33 |
| 12:20 - 12:40 | Appleclim project: innovative approaches to develop apple cultivars adapted to the Southern-Brazilian climatic conditions. | P.R.D. de Oliveira, L.F. Revers*, H.P. dos Santos, V. Quecini, F. Denardi, and L. Consoli | page 34 |
| 12:40 - 13:00 | Interspecific hybridization in sweet and sour cherry breeding.   | M. Schuster*, C. Grafe, E. Hoberg, and W. Schütze   | page 35 |
| 13:00 - 13:20 | Trends of apple breeding programs in Korea.  | D. Kim*, S.-I. Kwon, M.-J. Kim, S. Heo, J.H. Hwang, H. Shin, and Y.-U. Shin               | page 36 |

13:20 - 14:20

**Lunch**

**Oral session: Management and research on genetic resources**

**Chairs: R. Socias i Company and A.A. Przybyła**

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|---------------|--|--|---------|
| 14:20 - 14:40 | A catalog of molecular diversity of <i>Prunus</i> germplasm gathered from aligning NGS reads to the peach reference sequence: bioinformatic approaches and challenges. | S. Scalabrin, A. Policriti, F. Nadalin, S. Pinosio, F. Cattonaro, E. Vendramin, V. Aramini, I. Verde, D. Bassi, R. Pirona, L. Rossini, R. Testolin*, and M. Morgante | page 39 |
| 14:40 - 15:00 | Development, characterization and use of microsatellite markers for germplasm analysis in date palm ( <i>Phoenix dactylifera</i> L.).                                  | H. Arabnezhad, M. Bahar*, H.R. Mohammadi, and M. Latifian  | page 40 |
| 15:00 - 15:20 | Testing different approaches to construct olive ( <i>Olea europaea</i> L.) core subset suitable for association genetic studies.                                       | A. Elbakkali*, H. Haouane, P. Van Damme, and B. Khadari  | page 41 |

15:20 - 15:50

**Break**

**Oral session: Fruit crop physiology - reproductive biology**

**Chairs: T. den Nijs and M. Korbin**

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|---------------|--|----------------------------------|---------|
| 15:50 - 16:10 | Evaluation of citrus seed tolerance to desiccation and liquid nitrogen storage: towards citrus germplasm cryopreservation. | F. Luro*, I. Tur, and S. Dussert | page 45 |
|---------------|--|----------------------------------|---------|

16:10 - 16:30	Quantitative and qualitative expression of self-compatibility in almond.	R. Socias i Company*, À. Fernández i Martí, O. Kodad, and J.M. Alonso	page 46
16:30 - 16:50	Stigmatic receptivity limits fruit set in almond under warm climates.	O. Kodad, Z. Messaoudi, A. Mamouni, M. Lahlou, and R. Socias i Company*	page 47

16:50 - 18:20

**Poster session**

18:20

Departure to hotels

**Tuesday**

**13 September**

07:45

Departure from the Ibis Hotel

**Oral session: Fruit crop physiology - vegetative properties**

**Chairs: D. Dekena and F. Brandi**

09:00 - 09:20	Ethrel application shortens the juvenile phase of apple seedlings.	E. Żurawicz* and M. Lewandowski	page 51
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09:20 - 09:40	A genetic study of the vegetative and reproductive development in a F1 progeny 'Olivière' x 'Arbequina': toward a better understanding of olive tree ontogeny.	I. Ben Sadok*, G. Garcia, N. Moutier, F. Dosba, B. Khadari, and E. Costes	page 52
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09:40 - 10:00	Expression of the 4-coumarate: CoA ligase gene family in compatibles and incompatibles <i>Prunus</i> grafts.	I.S. Pereira, J.C. Fachinello, L.E.C. Antunes, P. Errea, R.S. Messias, and A. Pina*	page 53
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10:00 - 10:30

**Break**

**Oral session: Genetic and genomic studies of fruit quality**

**Chairs: H. Nybom and A. Pina**

10:30 - 10:50	Genetic relationships among fruit quality traits in red-fleshed apples.	R.K. Volz*, S. Kumar, D. Chagné, R. Espley, and A.C. Allan	page 57
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10:50 - 11:10	Biochemical composition and antioxidant activity of fruit crops.	N.I. Savelyev*, V.N. Makarov, A.N. Jushkov, N.N. Savelyeva, N.V. Borzykh, and M.J. Akimov	page 58
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11:10 - 11:30	Cloning and characterization of apple lipoxygenase (LOX) genes putatively involved in the formation of fruit flavour volatiles.	J. Vogt*, D. Schiller, W. Schwab, and F. Dunemann	page 59
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11:30 - 11:50	Diversity analysis of citrus fruit pulp acidity and sweetness: toward to understand the genetic control of the fruit quality parameters.	F. Luro*, J. Gatto, G. Costantino, and O. Pailly	page 60
11:50 - 12:10	Genetic study on fruit storage life in disconnected factorial mating population of <i>Actinidia chinensis</i> (kiwifruit).	C. Cheng* and S. Day	page 61
12:10 - 12:30	Preliminary results for inheritance of blush in pear cross combinations.	J.P. Human	page 62
12:30 - 12:50	Assessment of <i>Mal d 1</i> genes diversity in contemporary and historical Polish apple cultivars.	K.L. Bokszczanin*, A.A. Przybyła, and M. Filipecki	page 63
12:50 - 13:50	<p><b>Lunch</b></p> <p><b>Oral session: Biotechnology and molecular breeding</b></p> <p><b>Chairs: M. Kellerhals and S. Tartarini</b></p>		
13:50 - 14:20	Genetic engineering as a breeding tool in fruit crops. (invited lecture)	M.-V. Hanke	
14:20 - 14:40	Cisgenesis fits in the toolkit of a modern fruit breeder.	T. den Nijs, H. Schouten, and F. Krens	page 67
14:40 - 15:00	Heat-shock regulated excision of the <i>nptii</i> marker gene in transgenic apple ( <i>Malus × domestica</i> Borkh.).	K. Herzog, J. Würdig*, H. Flachowsky, H.B. Deising, and M.-V. Hanke	page 68
15:00 - 15:20	Introgression of a major quantitative trait locus for fire blight resistance using early flowering transgenic apple trees.	P.-M. Le Roux*, H. Flachowsky, M.-V. Hanke, C. Gessler, and A. Patocchi	page 69
15:20 - 15:40	Trans-generic <i>S</i> allele in apple.	K.L. Bokszczanin, A.A. Przybyła*, and A. Palucha	page 70
15:40 - 16:10	<p><b>Break</b></p> <p><b>Oral session: Biotechnology and molecular breeding</b></p> <p><b>Chairs: V.G. Bus and M. Kellerhals</b></p>		
16:10 - 16:40	FruitBreedomics: a new EU-project to improve fruit breeding efficiency. (invited lecture)	F. Laurens	page 71
16:40 - 17:00	Implementation of genomic selection in apple breeding.	S. Kumar*, D. Chagnè, M.C.A.M. Bink, R.K. Volz, R.N. Crowhurst, and C.J. Whitworth	page 72



17:00 - 17:20	The SA-ARC processing peach breeding programme: introducing molecular techniques.	W-M. Pieterse	page 73
17:20 - 17:40	A strategy for genetic mapping of polyploid kiwifruit ( <i>Actinidia</i> ) species.	P. Datson*, D. Mertton, G. Tsang, K. Manako, L. Fraser, and M. McNeilage	page 74
17:40 - 18:00	Targeted development and mapping of functional molecular markers in an apple rootstock ( <i>Malus pumila</i> ) mapping progeny.	L. Antanaviciute, F. Fernández-Fernández, J.M. Dunwell, N.H. Battey, and D.J. Sargent*	page 75
18:00 - 19:30	<b>Poster session</b>		
19:30	Departure to the Ibis Hotel		

### Wednesday 14 September

	Field trip		
07:15	Departure from the Ibis Hotel		
07:30	Departure from the Ikar Hotel		
20:00	Arrival to the Experimental Orchard in Wilanów		
22:00	Departure to hotels		

### Thursday 15 September

07:45	Departure from the Ibis Hotel		
	<b>Oral session: Genetic and genomic studies of resistance</b> <b>Chairs: F. Laurens and A. Patocchi</b>		
09:00 - 09:20	Progress in pyramiding disease resistances in apple breeding.	M. Kellerhals*, L. Franck, I. Baumgartner, J.E. Frey, and A. Patocchi	page 79
09:20 - 09:40	Observations on scab resistance in interspecific pear seedling families.	V. Bus*, L. Brewer, and Ch. Morgan	page 80
09:40 - 10:00	Development of a contig in apricot containing the PPV resistance locus.	J.M. Soriano, E. Zuriaga*, J. Martínez-Calvo, C. Romero, T. Zhebentyayeva, A.G. Abbott, and M.L. Badenes	page 81
10:00 - 10:20	A candidate gene of <i>Malus x robusta</i> 5 for breeding towards fire blight resistance.	J. Fahrenttrapp*, G. AL Broggini, M. Kellerhals, M. Malnoy, A. Peil, K. Richter, and C. Gessler	page 82
10:20 - 10:40	Genetics of resistance to <i>Amphorophora idaei</i> in red raspberry.	F. Fernández-Fernández*, L. Antanaviciute, V.H. Knight, J.M. Dunwell, N.H. Battey, and D.J. Sargent	page 83
10:40 - 11:10	<b>Break</b>		

## Oral session: Genetic and genomic studies of resistance

**Chairs: F. Laurens and J. Blažek**

11:10 - 11:30	First results of the initiative “Monitoring of <i>Venturia inaequalis</i> virulences”.	A. Patocchi*, A. Auwerkerken, S. Masny, H. Nybom, D. Pierre-Henri, L. Tamm, R. Vávra, A. Peil, B.A. Padder, M. Lateur, and V. Bus	page 84
11:30 - 11:50	QTL mapping for resistance to fire blight using several strains resulting in different host-pathogen interactions.	Th. Wöhner*, I. Vogt, K. Richter, M.-V. Hanke, C. Gessler, G. Broggini, J. Fahrentrapp, T. Garcia-Libreros, A. Peil, and H. Flachowsky	page 85
11:50 - 12:10	cDNA-AFLP analysis of differentially expressed transcripts in partial resistant apple cultivar leaves infected by <i>Venturia inaequalis</i> .	H. Bastiaanse*, A. Pissard, D. Mingeot, Y. Muhovski, J.-M. Jacquemin, P. Lepoivre, and M. Lateur	page 86
12:10 - 12:30	Transcriptomic analysis of two <i>Prunus</i> genotypes differing in waterlogging response reveals the importance of ANP and hypoxia-associated oxidative response.	M.J. Rubio-Cabetas*, M.L. Amador, C. Pons, C.A. Marti, and A. Granell	page 87
12:30 - 13:30	<b>Lunch</b>		
13:30 - 15:00	<b>Poster session</b>		
15:00 - 15:30	Business meeting and closing of Symposium		
15:30 -	Departure to the Ibis Hotel		
18:30 -	Departure from the Ibis Hotel		
19:00 - 23:00	Farewell dinner		
23:00	Departure to the Ibis Hotel		

## POSTER SESSIONS

**Monday**

**12 September**

16:50 - 18:20	<b>Poster session</b>		
16:50 - 17:20	<b>SESSION: Fruit breeding programmes</b>		
<b>Poster number</b>	<b>Moderators of discussion: M. Toth and E. Żurawicz</b>		
1	Selection for future on peach culture ( <i>Prunus persica</i> L. [Batsch]) in Romania.	A. Baciu*, I. Botu, S. Cosmulescu, M. Gruia, and L. Dumitru	page 93
2	Utility of wild germplasm in olive breeding.	T. Klepo, R. De la Rosa, L. León, and A. Belaj*	page 94

3	Breeding of resistant strawberry cultivars for organic fruit production: preliminary results with <i>Botrytis cinerea</i> .	M. Bestfleisch*, M. Höfer, K. Richter, M.-V. Hanke, E. Schulte, and H. Flachowsky	page 95
4	Breeding of highbush blueberry in Lithuania.	L. Česonienė* and R. Daubaras	page 96
5	Peach breeding programme for new and different traits. Pomological and phenological data analysis with a ranking method.	G. Cipriani*, L. Conte, D. Bevilacqua, A. Di Cintio, M. Terlizzi, and A. Sartori	page 97
6	Selection of European cranberry in Lithuania.	R. Daubaras* and L. Česonienė	page 98
7	Achievements in apple breeding at Zhengzhou Fruit Research Institute, CAAS.	G. Guo*, Y. Zhenli, Z. Hetao, and Z. Ruiping	page 99
8	Almond breeding in Iran and its achievements.	A. Imani	page 100
9	Trans-border pear breeding program for scab tolerance and novel traits.	M. Lateur*, J.-B. Rey, A. Rondia, M. Lascoste, and R. Stiévenard	page 101
10	Red pear breeding in China.	X. Li	page 102
11	Developing economically and culturally viable apple cultivars for the midwest United States: results from the Midwest Apple Improvement Association breeding program.	D.D. Miller	page 103
12	Hansabred – a new European strawberry breeding and research company.	K. Olbricht*, U. Gerischer, A. Ludwig, S. Kraege, S. Walpole, P. Walpole, B. Casas, A. Darbonne, A. Vissers, and H. Obers	page 104
13	Vf- scab resistant apple cultivars from 35-years apple breeding programme in Warsaw.	E. Pitera	page 105
14	Widening hazelnut ( <i>Corylus avellana</i> L.) genetic resources and selection criteria for the genetic improvement of phenological, pest resistance, and seed size traits.	G. Catarcione, D. Vittori, S. Bizzarri, E. Rugini*, and C. De Pace	page 106

17:20 - 17:50

**SESSION: New cultivars**  
**Moderators of discussion: C. R. Hampson**  
**and H. Flachowsky**

15	Determination of cluster characteristics and pomological properties of selected clones of Elhamra ( <i>Vitis vinifera</i> L.) grape variety.	A. Dardeniz*, M. Seker, K. Kahraman, G. Arda, and L. Türker	page 111
16	Better3fruit: commercial releases after 10 years.	I. De Wit*, A. Auwerkerken, and A. Van den Putte	page 112
17	Nectarine and brugnone cultivars obtained at Research Station for Fruit Growing Constanta – Romania.	L.-M. Dumitru* and A. Indreias	page 113
18	Assessment of cherry cultivars and selections in Latvia.	D. Feldmane*, S. Ruisa, and I. Samsone	page 114
19	Results of pollination studies of some new plum cultivars in Latvia	I. Grāvīte and E. Kaufmane	page 115
20	SPC 207: a new blush sweet cherry from Summerland	C.R. Hampson*, F. Kappel, R. MacDonald, and D.-L. McKenzie	page 116
21	Behaviour of some plum cultivars in the Center of Transylvania.	G. Roman, E. Hărșan*, M. Mărghitaș, St. Wagner, A.P. Somsai, and M. Giurgiu	page 117
22	Results concerning the behaviour of some rootstocks for peach tree in the nursery and orchard research trials.	A. Indreias* and L.M. Dumitru	page 118
23	‘Hongaram’: a red fruited, high aroma table grape.	J.H. Noh*, K.S. Park, H.K. Yun, Y.Y. Hur, S.J. Park, S.M. Jung, and H.S. Hawang	page 119
24	Productive value of some Polish scab-resistant apple cultivars grown on different rootstocks.	M. Lewandowski*, E. Żurawicz, and K. Rutkowski	page 120
25	New pear cultivars from Czech Republic.	F. Paprstein*, A. Matejicek, and J. Sedlak	page 121
26	Results of ten-year rootstock testing for apple cultivar Rubin on a fertile soil.	C. Piestrzeniewicz*, A. Sadowski, and R. Dziuban	page 122
27	New sour cherry cultivars from Dresden-Pillnitz.	M. Schuster*, C. Grafe, and B. Wolfram	page 123
28	Results of sweet cherry breeding in Dresden-Pillnitz.	M. Schuster*, C. Grafe, and H. Schmidt	page 124
29	Agrotechnical and biological properties of some sour cherry cultivars and their suitability for cultivation in Central Poland.	E. Szpadzik*, E. Jadczyk-Tobjasz, and B. Łotocka	page 125

30	New apple cultivars registered in Hungary: Artemisz, Hesztia and Rosmerta.	M. Tóth*, S. Kovács, G. Ficzek, and M. Hevesi	page 126
31	New apple rootstock selections from Estonia.	T. Univer*, N. Univer, and K. Tiirmaa	page 127
32	SD9238: a ‘real’ semi-dwarf germplasm of nectarine.	Z. Wang*, L. Niu, Z. Lu, G. Cui, and Y. Song	page 128
33	The effect of 1-MCP and controlled atmosphere on the quality and storability of U 7979 and ‘Mutsu’ apples.	K. Tomala*, K. Jeziorek, U. Ogłodzińska, T. Krupa, A. Sharafi, and M. Stepniewska	page 129
34	Growth and cropping of ‘Jonagored’ apple trees depending on rootstock and autumn nitrogen fertilisation.	D. Wrona* and W. Kowalczyk	page 130
17:50 - 18:20	<b>SESSION: Management and research on genetic resources (part 1)</b>		
	<b>Moderators of discussion: S. Tartarini and B. Gelvonauskis</b>		
35	Developing a core collection of olive ( <i>Olea europaea</i> L.) based on molecular markers (DARs, SSRs, SNPs) and agronomic traits.	A. Belaj*, M. del Carmen Dominguez-García, S. G. Atienza, N.M. Urdíroz, R. De La Rosa, Z. Satovic, A. Martín, A. Kilian, I. Trujillo, V. Valpuesta, and C. Del Río	page 133
36	Investigation of kolomikta kiwi ( <i>Actinidia kolomikta</i> ) phenotypic and genetic diversity.	L. Česonienė* and R. Daubaras	page 134
37	Cranberry bush <i>Viburnum opulus</i> – a new plant for horticulture.	R. Daubaras* and L. Česonienė	page 135
38	Conservation and use of fruit genetic resources in Lithuania.	B. Gelvonauskis*, J. Labokas, S. Žilinskaitė, D. Gelvonauskienė, and L. Česonienė	page 136
39	Establishment of first collection of Iranian <i>Rubus</i> germplasm and preliminary study of genetic diversity, pomological potential and nutritional value of the accessions.	A. Gharaghani*, S. Eshghi, S.H.A. Momeni, and Z. Keshavarz	page 137
40	Establishment of quince ( <i>Cydonia oblonga</i> Mill.) germplasm collection from various regions of Iran.	H. Abdollahi, M. Alipour, M. Khorramdelazad*, S. Mehrabipour, A. Ghasemi, M. Adli, D. Atashkar, and M. Akbari	page 138
41	Analysis of SSR marker polymorphism among Korean grapes.	Y.-J. Oh, J.-Y. Park, I.-C. Son, D. Kim*	page 139

42	Morphological characteristics and genetic relationships among Korean table grape cultivars.	Y.-J. Oh, J.-Y. Park, S.-I. Oh, J.H. Park, I.-C. Son, J.H. Noh, and D. Kim*	page 140
43	Yielding characteristics of <i>Rosa</i> taxa.	S. Kovács*, É. Szabóné Erdélyi, D. Szilágyi, and M. Tóth	page 141
44	A histological study on fruit skin and anther of plum ‘Soldam’, apricot ‘Harcot’ and plumcot (plum × apricot) ‘Harmony’.	E.Y. Nam*, S.K. Yun, J.H. Jun, I.K. Yoon, Y.U. Shin, and K.H. Chung	page 142
45	Evaluation of Canadian accessions of <i>Fragaria virginiana</i> Miller with regard to strawberry breeding.	A. Ludwig, M. Bartho, S. Kuss, C. Kuss, S. Plaschil, K. Olbricht*, and P. Scheewe*	page 143
46	Rescue of old sweet cherry cultivars.	F. Paprstein*, A. Matejicek, and J. Sedlak	page 144
47	Predictive modeling of species distributions: occurrences and environmental variables with fruit trees.	M.P. Errea, A. Pina*, M.T. Espiau, and P. Errea	page 145
48	Plant and fruit characteristics of <i>Arbutus unedo</i> L. and <i>Arbutus andrachnae</i> L. from the Highlands of Northwestern Turkey.	N. Ekinçi, A. Sakaldas, M. Seker*, and H. Ekinçi	page 146
49	Genetic diversity in an almond germplasm collection: application of a chemometric approach.	O. Kodad, G. Estopañán, T. Juan, and R. Socias i Company*	page 147

## Tuesday

**13 September**

**Poster session**

**SESSION: Genetic and genomic studies  
of fruit crop physiology**

**Moderators of discussion: M. Lateur  
and K.L. Bokszczanin**

18:00 - 19:30

18:00 - 18:30

50	Detection of pear <i>S</i> -alleles by setting up a revised identified systems.	F. Babaei*, H. Abdollahi, and M. Khorramdelazad	page 151
51	Performance of tree growth characteristics in selected progenies of columnar apple cultivars.	J. Blažek	page 152
52	Influence of plum rootstocks on the dynamic of dry matter in the annual shoots of cultivar ‘Victoria’.	D. Dekena*, I. Alsina, and J. Lepsis	page 153

- 53 Identification of self-incompatibility alleles in Iranian pear cultivars using PCR analysis. M. Bagheri and A. Ershadi\* page 154
- 54 Monogenic sign control of a pear compact habit. V.S. Girichev page 155
- 55 The effect of IBA, NAA hormones on rooting of Russian olive (*Elaeagnus angustifolia*) cutting. V. Jajarmi page 156
- 56 Isolation and expression analysis of gibberellin 20-oxidase gene in dwarf rootstock of pear 'Zhongai 1'. S. Jiang\*, L. Xuan, C. Ou, F. Wang, Z. Wang, F. Cheng, and Z. Li page 157
- 57 Identification of genes that vary their expression during bud dormancy release in ten peach varieties having low and medium chilling requirements. C. Leida\*, M.L. Badenes, and G. Ríos page 158
- 58 Optimization of strawberry pollen culture and its maintenance of pollen germination capacity. S. Piripireivatlou\*, A. Imani, and S.H. Masomi page 159

18:30 - 19:00

**SESSION: Genetic and genomic studies  
of fruit quality**  
**Moderators of discussion: R.K. Volz  
and F. Fernández-Fernández**

- 59 A novel anthocyanin gene in domestic apple. K.R. Tobutt, C. Bester\*, and J.P. Human page 163
- 60 Genetic, biochemical and molecular studies of carotenoid accumulation and aromatic compound emission in white- and yellow-fleshed peach genotypes. F. Brandi\*, A. Liverani, D. Giovannini, G. Bianchi, V. Papa, M. Buccheri, F. Mourgues, and C. Rosati page 164
- 61 A predictive genetic test for apple "fresh taste" provides strategies for improved breeding and fruit handling. S. Verma, Y. Guan, K.M. Evans\*, J. McPerson, E. van de Weg, J. Luby, S.K. Brown, and C. Peace page 165
- 62 *In silico* genome wide search for putative genes of the antioxidant key enzymes involved in the physiological elimination of ROS. P. Irisarri\*, P. Errea, and A. Pina page 166
- 63 Integrated omics analysis of spontaneous mutant of pear setting giant fruits. K. Isuzugawa\*, T. Takashina, Y. Takahashi, T. Hanada, A. Oikawa, R. Nakabayashi, F. Matsuda, S. Yamaguchi, H. Mori, and K. Shiratake page 167



- 64 Analysis of suppression subtractive hybridization for differently expressed genes between ‘Redfield’ and ‘Greensleeves’ apple fruits. S.E. Han, H.E. Lee, S. Heo, Y.-J. Oh, D. Kim\* page 168
- 65 Evaluation of nutraceutical content in fruit of 13 highbush blueberry cultivars. T. Krupa page 169
- 66 Use of NIR spectroscopy for breeding apples of quality and nutritional features. A. Pissard, P. Dupont, V. Baeten, G. Sinnaeve, J-M. Romnee, A. Mouteau, and M. Lateur\* page 170
- 67 Fruit and oil characteristics of advanced selections from an olive breeding program. P.J. León\*, N. Talhaoui, L. León, L. Velasco, B. Pérez-Vich, and R. De la Rosa page 171
- 68 Investigation of volatile constituents in some promising local peach and nectarine genotypes using HS-SPME technique by GC-MS. M. Seker, E. Gur, and N. Ekinçi page 172
- 69 Kernel chemical composition of the walnut genetic resources grown in the High Atlas Mountains of Morocco. O. Kodad, J.P. Wathelet, G. Estopañan, T. Juan, R. Socias i Company\*, and M. Sindic page 173
- 70 Cloning and expression patterns of ten cDNAs associated with pear fruit (*Pyrus pyrifolia*) during the development and ripening stage. R. Wang\*, X.N. Zhang, S.L. Yang, J. Zhu, M. Wang, and N.N. Zhao page 174
- 71 Lignin metabolism in russet fruit skin of a pear mutant and cloning of its correlated gene. H. Wei\*, Y. Zhen-feng\*, J. Bing, L. Xiao-feng, L. Xue, and Z. Li-wu page 175
- 72 Influence of calcium content on lipoxygenase activity of apple cultivars ‘Gala’ and ‘Sampion’. M. Wińska-Krysiak page 176

19:00 - 19:30

**SESSION: Biotechnology, marker development and molecular breeding**

**Moderators of discussion: M.-V. Hanke and D. Kim**

- 73 Development of novel microsatellite markers from an enriched genomic library in *Pistacia vera* L. S. K. Zenoozi\*, M. Mardi, I. Tabatabaei, S.M. Pirseyedi, M. Farsi, M. Zeinalabedini, M.A. Ebrahimi, and S.M.K. Nekoui page 179
- 74 Detection and validation of SNPs and a SFP in chloroplast *Prunus* species. B. Bielsa, D. Jiwan, A. Dhingra, A. Fernandez-Marti, and M.J. Rubio-Cabetas\* page 180



75	SNP development on a resequenced peach genotype for mapping and QTL analysis.	S. Tartarini*, L. Dondini, R. Paris, G. Pagliarani, S. Venturi, S. Sansavini, F. Gennari, E. Marasco, V. Mantovani, R. Pirona, I.P. Cruz, L. Rossini, S. Foschi, S. Scalabrin, F. Cattonaro, D. Bassi, and I. Verde	page 181
76	Development of SNP markers for marker - assisted selection in pear.	S. Terakami*, C. Nishitani, and T. Yamamoto	page 182
77	Heat-induced precocious flowering of apple plants containing the <i>FLOWERING LOCUS T</i> gene from poplar ( <i>Populus trichocarpa</i> ).	S. Wenzel, H. Flachowsky*, S.H. Strauss, and M.-V. Hanke	page 183
78	DNA markers developed from genome sequencing analysis in Japanese pear ( <i>Pyrus pyrifolia</i> ).	T. Yamamoto*, S. Terakami, S. Moriya, K. Kurita, H. Kanamori, Y. Katayose, T. Saito, and C. Nishitani	page 184
79	Profiling transcripts of defense related response genes from <i>Vitis coignetiae</i> native to Korea.	H.K. Yun*, S.Y. Ahn, Y.J. Choi, S.H. Kim, and Y.Y. Hur	page 185
80	Deep re-sequencing of apricot genome toward genome-wide association analysis in stone fruit species.	T. Zhebentyayeva, C. Dardick, J.M. Soriano, E. Zuriaga*, M.L. Badenes, and A.G. Abbott	page 186

## Thursday

**15 September**

**Poster session**

**SESSION: Genetic and genomic studies  
of biotic stress**

**Moderators of discussion: T. Yamamoto  
and A.A. Przybyła**

13:30 - 15:00

13:30 - 14:15

81	Is tolerance to <i>Penicillium expansum</i> associated with ripening date and fruit firmness in apple?	M. Ahmadi-Afzadi*, I. Tahir, J. Sehic, and H. Nybom	page 189
82	Application of P gene donors in breeding of black currant resistant to gall-mite.	V. Bendokas*, I. Mazeikiene, D. Baniulis, V. Stanys, and T. Siksnianas	page 190
83	Increasing importance of pests on highbush blueberry plantations in Poland.	A. Chorąży	page 191
84	Screening of apple cultivars for resistance to European canker, <i>Nectria galligena</i> .	L. Garkava-Gustavsson*, A. Zborowska, J. Sehic, M. Rur, H. Nybom, E. van de Weg, and A. Holefors	page 192

85	Diversity of resistance to <i>Monilinia laxa</i> and spring frost in sour cherry germplasm.	D. Gelvonauskiene*, B. Frercks, I. Stepulaitiene, and V. Stanys	page 193
86	Inheritance of partial resistance to apple scab in 10 apple progenies.	C.R. Hampson	page 194
87	Comparative studies on the behavior of hybrid apple progenies resistant to <i>Venturia inaequalis</i> under field and "in vitro" conditions.	L. Ion*, D. Hoza, Gh. Petre, V. Petre, C. Moale, V. Lupu, and T. Neagu	page 195
88	Evaluation of resistance to sharka (plum pox) in some Romanian apricot progenies.	L. Ion	page 196
89	Improvement of Romanian old varieties of apple as genitors in obtaining hybrids progenies with genetic resistance to <i>Venturia inaequalis</i> .	L. Ion	page 197
90	Improvement of Romanian rootstocks genotypes to restrict the PPV infection in some apricot progenies.	L. Ion*, D. Hoza, C. Moale, I. Zagrai, L. Zagrai, V. Lupu, and S. Preda	page 198
91	Apple cultivars and hybrids resistant to <i>Nectria canker</i> as initial material for breeding.	Z.A. Kazlouskaya* and Y.G. Marchuk	page 199
92	Resistance of apple hybrids to scab and powdery mildew in the nursery.	Z.A. Kazlouskaya*, T.A. Hashenka, and V.V. Vaseha	page 200
93	The HTS-based study on transcriptome of strawberry ( <i>Fragaria x ananassa</i> ) infected with <i>Verticillium dahliae</i> .	M. Korbin*, T. Jęcz, U. Lavi, L. Farinelli, M. Osteras, N. Vincent, and M. Dudkiewicz	page 201
94	Mapping of quantitative trait loci for fire blight resistance in the apple cultivar 'Rewena'.	P-M. Le Roux*, A. Peil, K. Richter, C. Gessler, and A. Patocchi	page 202
95	Occurrence of sooty blotch on apple selections resistant to scab.	E. Mirzwa-Mróż*, M. Wińska-Krysiak, R. Dziecioł, and E. Pitera	page 203
96	Genetic mapping of the gene for susceptibility to <i>Alternaria blotch</i> in apple.	S. Moriya*, S. Terakami, H. Iwanami, T. Haji, K. Okada, T. Yamamoto, and K. Abe	page 204
97	The occurrence of the highbush blueberry viruses in Poland.	E. Paduch-Cichal*, E. Kalinowska, M. Chodorska, B. Nowak, K. Sala-Rejczak, M. Szyndel, Z. Gajewski, and K. Tomala	page 205

98	Exploring genomic organisation of scab resistance in pear.	D. Leforestier, C. Denancé, P. Guérif, M. Tellier, L. Bouvier, M. Chevalier, Y. Lespinasse, C.E. Durel, and L. Perchepped*	page 206
99	Development of a genetic linkage map for identification of molecular markers associated with resistance to bacterial spot ( <i>Xanthomonas arboricola</i> pv. <i>pruni</i> ) in peach.	N. Yang, G. Reighard*, D. Ritchie, W. Okie, and K. Gasic	page 207
100	<i>Malus</i> sp. – <i>V. inaequalis</i> interaction characteristics among accessions of apple genetic resources.	S. Sikorskaite*, D. Gelvonauskiene, V. Bendokas, V. Stanys, and D. Baniulis	page 208
101	Mapping QTL resistance against <i>Xanthomonas arboricola</i> pv. <i>pruni</i> in apricot.	D. Socquet-Juglard*, D. Christen, B. Duffy, C. Gessler, and A. Patocchi	page 209
102	Inheritance of anthracnose ( <i>Sphaceloma ampelinum</i> ) resistance in grapevine.	P. Tantasawat*, O. Poolsawat, and S. Wongkaew	page 210
103	Evaluation of local apple varieties in northern Italy as sources of scab resistance.	M. Komjanc, E. Zini*, M. Troglio, R. Osler, R. Velasco, and P. Ermacora	page 211
14:15 - 15:00	<b>SESSION: Management and research on genetic resources (part 2)</b> <b>Moderators of discussion: H. Nybom and R. Testolin</b>		
104	Genetic diversity between pear cultivars in Iran using simple sequence repeats marker (SSR).	J. Erfani, H. Abdollahi, A. Ebadi, R. Fattahi, M. Khorramdelazad, and F. Babaei*	page 215
105	Study of genetic diversity of selected Iranian almond germplasms using fluorescent-AFLP markers.	M. Farsi*, M. Zeinalabedini, A. Imani, B. Nakhoda, P. Majidian, M. Dabbab, and M. Mardi	page 216
106	Analysis linked SSR markers associated to gene controlling of traits in quince ( <i>Cydonia oblonga</i> Mill.).	M. Khorramdelazad*, H. Abdollahi, and M. Alipour	page 217
107	Identification of synonyms by old Hungarian apple cultivars using morphological and molecular markers.	I. Király*, O. Nagyistván, É.S. Erdélyi, and M. Tóth	page 218
108	SSR marker based fingerprinting for sour cherry ( <i>Prunus cerasus</i> ) genetic resources identification and management.	G. Lācis* and I. Kota	page 219

- 109 Genetic variability and relationship of the heritage peach varieties of Emilia Romagna (Italy). A. Leone\*, M.T. Dettori, A. Liverani, S. Sirri, I. Verde, and D. Giovannini page 220
- 110 Characterization of microsatellite marker loci in Lithuanian pear (*Pyrus communis*) cultivars. V. Lukoseviciute\*, D. Gelvonauskiene, G. Staniene, D. Baniulis, A. Sasnauskas, V. Stanys, and R. Rugienius page 221
- 111 DNA fingerprinting of new Iranian apricots released from breeding programs. M. Dabbab, M. Zeinalabedini, J. Dejampour, P. Majidian\*, B. Nakhoda, M. Farsi, M. Mardi, and A. Imani page 222
- 112 Evaluation of genetic relationships of some apricot cultivars and genotypes using fluorescent-AFLP markers. P. Majidian\*, M. Zeinalabedini, J. Dejampour, B. Krska, B. Nakhoda, M. Dabbab, M. Farsi, M. Mardi, and A. Imani page 223
- 113 The survey of genetic diversity & population structure analysis of Iranian sweet pomegranate (*Punica granatum* L.) germplasm using SSR markers. S.M. Mousavi Derazmahalleh\*, M. Mardi, S.H. Marashi, S. Malekzadeh, S.M. Khayam Nekoui, M. Zeynalabedini, M. Kazemi, and T. Roodbar Shojaie page 224
- 114 The study of genetic diversity and relationships among some wild *Prunus* species using vegetative traits and molecular markers. K. Nikoumanesh\*, A. Ebadi, and M.R. Fattahi page 225
- 115 More harmonization needed for DNA-based identification of fruit tree germplasm. J. Sehic, L. Garkava-Gustavsson, and H. Nybom\* page 226
- 116 Genetic identification of 'Bistrica' and her synonyms 'Požegača' and 'Hauszwetsche' (*Prunus domestica* L.) using SSRs. D. Halapija Kazija, P. Drkenda, S. Šimon, M. Žulj, F. Gaši, D. Nikolić, P. Vujević, T. Jelačić, B. Milinović, M. Kurtović, and I. Pejić\* page 227
- 117 Accuracy of the genetic analysis for early selection of hazelnut (*Corylus avellana* L.) genetic resources is increased by *in vitro* micropropagation of zygotic embryos. P. Gutiérrez Pesce, G. Catarcione, E. Rugini\*, and C. De Pace page 228
- 118 Application of chloroplast DNA in determination of *Punica granatum* phylogeny. B.E. Sayed Tabatabaei\* and E. Keyvanifar page 229
- 119 Genetic diversity of Iranian pomegranate (*Punica granatum* L.) cultivars using microsatellite markers. B. Sharifnabi\*, S. Ebrahimi, and B.E. Sayed Tabatabaei page 230

120	Evaluation of genetic variability inside an Italian apple germplasm collection.	L. Dondini, P. De Franceschi, W. Liang, R. Paris, F. Gennari, S. Sansavini, and S. Tartarini*	page 231
121	Identification of apple sport mutants of 'Antonovka' by molecular methods.	O.Yu. Urbanovich*, P.V. Kuzmitskaya, Z.A. Kazlouskaya, and N.A. Kartel	page 232
122	A genetic identification method for raspberry ( <i>Rubus idaeus</i> ) and blackberry ( <i>Rubus fruticosus</i> ) cultivars by microsatellites (SSR).	C. Fritzmann, G. Muster, and H. Xuan*	page 233
123	Identifying quince cultivars by means of apple- and pear microsatellites.	D. Spann, M. Neumüller, and H. Xuan*	page 234
124	Evaluation of honeysuckle ( <i>Lonicera</i> sp. L.) germplasm collection using DNA markers.	D. Naugzemys, S. Zilinskaitė*, A. Skridaila, V. Kleizaitė, and D. Zvingila	page 235
125	Primarily identification of pear variants and analysis of their genetic relationship by SRAP markers	Z. Li-wu*, X. Ao-jing, J. Bing, H. Wei, and Y. Zhen-feng	page 236
15:00 - 15:30	Business meeting and closing of Symposium		
15:30	Departure to hotels		
18:30	Departure from hotels		
19:00 - 23:00	Farewell dinner		



# Abstracts





# Oral sessions



**Oral session**

**BREEDING PROGRAMMES  
AND NEW CULTIVAR DEVELOPMENT**



## **APPLE BREEDING AND GENETIC CONTROL OF THE MAIN AGRONOMIC, RESISTANCE AND FRUIT QUALITY TRAITS**

**S. Sansavini\* and S. Tartarini**

Department of Fruit Trees and Woody Plant Sciences, Bologna University, Viale Fanin 46, 40127, Bologna, Italy

The main objectives being pursued by today's fruit breeding programs include quality, resistance to biotic stresses, environmental adaptability and enhanced bearing (i.e. growth habit, fruiting limbs, yield efficiency and stability, and so forth). These objectives are mainly driven by the new world trends of consumer demand that have a wide market range from sweet and aromatic apples, to sour and firm, crisp and juicy flesh, with very varied skin color, all in any case of lengthy storability and good shelf-life. The traditional phenotypic selection of parents and seedlings is starting to be implemented by the use of the available genomic tools like genetic maps and markers linked to qualitative and quantitative traits. A further step towards the optimization and real application of the marker-assisted selection in apple is promised by the recent publication of the whole genome apple sequence obtained by an International project coordinated by FEM-IASMA (S. Michele all'Adige, Trento, Italy). In fact the availability of the whole genome sequence represent a revolution in the development of new genomic tools making much easier the development of markers tightly linked to traits of interest but also towards the identification of genes controlling specific traits. These genomic advances are going to be not restricted to apple but also to pear due to the known synteny between the two genomes. The easier identification of apple gene sequences responsible for specific traits through genome walking combined with the possibility to obtain cisgenic apples should also remove or reduce the public opposition to GM plants opening the way to obtain improved commercial GM varieties.

## **40 YEARS OF AUSTRALIAN NATIONAL APPLE BREEDING PROGRAM**

**F. Shan**

Department of Agriculture and Food Western Australia, 3 Baron Hay Court, South Perth, Western Australia 6151

It has been 40 years since the commencement of Australian National Apple Breeding Program (ANABP) in 1972. It has been funded mainly by the Dept of Agriculture and Food Western Australia. Since 1989 there has intermittently been investment from the Federal Government through Horticulture Australia Ltd (HAL). ANABP's breeding objective is to produce novel apple varieties and maximize their benefit to the Australian Apple Industry. Quality has been the priority. The new variety needs to have long storage life and be suitable for production in Australian environments with long hot and dry summer and mild winters. ANABP is a conventional breeding program by sexual hybridisation to generate hybrid seeds. The hybrids will go thru a series of rigorous assessments, namely stage 1 to stage 3, which constitutes the ANABP breeding process. The evaluation in these stages aimed to both describe a potential variety's characteristics and points of differentiation, and to ensure that it performs against a range of quality standards. Selections whose performance and specific characteristics are considered to offer an opportunity for Australian apple industry are then recommended as a commercial candidate for release. In the breeding process the last stage, stage 4, is commercialization, involving the strategic release and commercial development of a new variety. Up to 2010, total 311 crosses have been made and 166156 seedlings planted in the field. Four varieties have been released including Cripps Pink (Pink Lady<sup>TM</sup>), Cripps Red (Sundowner<sup>TM</sup>), Big Time and Western Dawn (Enchanted<sup>®</sup>). PBR protection has been granted to Western Tang. Full PBR application for a dark apple ANABP 1 is under progress and is targeted to be released in 2012. There are number of advanced selections which are still under assessment at various stages. The strategy changes, technique development, issues and the future of the breeding program are discussed.

## A NEW ALMOND BREEDING PROGRAM IN TURKEY

**I. Acar<sup>1\*</sup>, S. Arpacı<sup>1</sup>, H.S. Atlı<sup>1</sup>, S. Kafkas<sup>2</sup>, S. Eti<sup>2</sup>, S. Caglar<sup>3</sup>, and A. Yılmaz<sup>1</sup>**

<sup>1</sup>Pistachio Research Institute, 27060, Sahinbey, Gaziantep, Turkey

<sup>2</sup>Cukurova University, Fac. of Agriculture, Dept of Horticulture, Balcalı, Adana, Turkey

<sup>3</sup>Sutcu Imam University, Fac. of Agriculture, Dept of Horticulture, Kahramanmaraş, Turkey

Turkey has favorable ecological conditions especially lengthwise Aegean, Mediterranean and Southeastern Regions for almond production. Almost entire of the domestic cultivars in Turkey are stone almond type and they are self-incompatible. For this reason, whole of the certificated almond saplings are produced by using foreign originated cultivars, as native varieties unable to attract the attentions in markets. The objective of this study is breeding of the late flowering and self-fertile almond cultivar/s by hybridization of some domestic cultivars and self-fertile foreign almond varieties. Self-compatible Lauranne, Guara and Moncayo cultivars were hybridized with self-incompatible Gulcan 1, Gulcan 2, Akbadem and Nurlu cultivars in 2009. After the hybridization 6.116 seeds and 4.184 seedlings (average germination 68.4%) were obtained. Self-compatible seedlings have been determined by S allele PCR method. The self-compatible hybrids have been planted in Turkish Pistachio Research Institute by 2 m x 1 m distances on winter 2011. Several characteristics, such as leafing date, vigour, growth habit and branching will be observed during the 2011. Blooming date, early cropping and nut traits will be assessed during the subsequent years.

## APPLE BREEDING AND GENETIC RESOURCES IN LATVIA

**L. Ikase\* and G. Lacis**

Latvia State Institute of Fruit-Growing, Graudu 1, Dobeles, LV-3701, Latvia  
e-mail: laila.ikase@lvai.lv

Latvia belongs to the northern region of apple growing with specific demands for winter-hardiness and length of the fruit maturing period. Fluctuating winter and spring temperatures and relative abundance of precipitation add to the challenges. The apple assortment in Latvia was initially formed by cultivars from Germany, Russia, Poland-Lithuania and Scandinavia. Until the 20th century, open-pollinated seedlings were the only source of important local cultivars like 'Baltais Dzidrais' ('Transparente Blanche'). In 1950ties and early 21st century expeditions were organized collecting and evaluating landraces, clones, amateur cultivars and the best chance seedlings. At present, the genetic resource collection includes about 260 accessions of Latvian origin, the base collection (109) duplicated at Latvia State Institute of Fruit-Growing (LSIFG) and Pure Horticulture Research Centre. Genetic analysis was conducted for 109 cultivars, making clearer their relationships and parentages. About 400 accessions are in the stage of evaluation, their use in breeding defined as the priority. Controlled crosses were started in 1950ties using cultivars best adapted to Latvian climate and later involving donors of compact growth, columnar habit and scab resistance genes *Vf* and *Vm*. Their breeding work was continued at LSIFG and Pure HRC. Several of the resulting cultivars are grown commercially ('Iedzenu', 'Forele', 'Agra') or are promising for commercial orchards ('Eksotika', 'Daina' etc.). The latest releases include scab resistant (*Vf*) cultivars 'Dace', 'Edite', 'Gita', 'Ligita', 'Roberts' and columnar apples 'Inese' (*Vf*), 'Duets', 'Baiba', 'Uldis', 'Zane'. The present breeding program at LSIFG is aimed at combining traits important for the development of competitive commercial apple growing in Latvia – improved fruit quality and prolonged storage, complex resistance to fungal diseases (scab, *Nectria* canker, mildew, storage rots etc.) and physiological disorders, regular yields with self-thinning fruits, easy tree habit and winter-hardiness.



## **APPLE BREEDING IN THE PACIFIC NORTHWEST**

**K.M. Evans**

Washington State University Tree Fruit Research and Extension Center, 1100 N. Western Ave, Wenatchee, WA 98801, USA

The Washington State University (WSU) breeding program was started in 1994 with the aim of producing new varieties especially suited to the main production areas of the state. The program has targeted improving quality, particularly texture, as well as good appearance and storability. The program currently has some 12,000 fruiting trees in the phase 1 assessment plots and 62 advanced selections in phase 2 replicated plantings at three different sites. Eight elite selections are being assessed in phase 3 grower trials at four different sites. The first release from the program was ‘WA 2’ in 2009; ‘WA 5’ was released in 2010. The program is currently implementing DNA-informed breeding. Marker-assisted parent selection has been used for several years and high through-put systems have recently been implemented for seedling selection, however there are still very few markers that are linked to fruit quality. The WSU apple breeding program is one of the demonstration programs for the USDA SCRI-funded RosBREED project which has ‘implement marker-assisted breeding in core RosBREED breeding programs’ as one of the major targets. Phenotypic and genotypic data is currently being collected within this project with a view to the development of new robust DNA markers linked to socio-economically important fruit quality traits.

## APPLECLIM PROJECT: INNOVATIVE APPROACHES TO DEVELOP APPLE CULTIVARS ADAPTED TO THE SOUTHERN-BRAZILIAN CLIMATIC CONDITIONS

P.R.D. de Oliveira<sup>1</sup>, L.F. Revers<sup>1\*</sup>, H.P. dos Santos<sup>1</sup>, V. Quecini<sup>1</sup>, F. Denardi<sup>2</sup>, and L. Consoli<sup>3</sup>

<sup>1</sup>Embrapa Uva e Vinho, Bento Gonçalves, Brazil

<sup>2</sup>Epagri, Estação Experimental de Caçador, Caçador, Brazil

<sup>3</sup>Embrapa Trigo, Passo Fundo, Brazil

In the Brazilian apple production regions, usually occurs insufficient chilling exposure as well as temperature fluctuations during winter dormancy. These unfavorable climatic conditions tend to be intensified considering to global warming predictions. The purpose of the project is to gather data and knowledge about how bud dormancy is genetically and physiologically controlled and to generate biotechnological tools applied to conventional breeding in order to accelerate the generation of adapted cultivars. Employing a multidisciplinary approach, bud samples treated for different regimes of temperatures are being used for global gene expression analysis, 2D proteomic analysis followed by mass spectrometry, LC-MS-MS metabolic profiling and modeling of the dormancy process under Brazilian climatic conditions. High-throughput regeneration and gene-delivery systems to apple scion and rootstocks are also being developed. *In silico* analyses and linkage mapping data to identify candidate genes for important processes, such as growth and developmental transitions control, are also underway. In order to develop new apple varieties displaying low chilling requirements and disease resistance, 15.000 to 20.000 seeds are being obtained bi-annually. Following diseases inoculations, the remainder seedlings populations are evaluated in the field for climatic adaptation, vigor and juvenility. The pre-selections grafted on M-9 rootstock are being evaluated for red-skin color, sweet flavor and long storage capacity as the main attributes for fruit quality. From the 64 selections under evaluation, 45 are being tested on a regional network collection scheme, to allow selection for good local climatic adaptation, disease resistance, yield and fruit quality. During the project, the new variety Monalisa was released, a low chilling and multi-resistant one, carrying the *Vf* scab resistance allele, high resistance to *Colletotrichum gloeosporioides* and to European red spider mite and good tolerance to both mildew and bitter rot. This cultivar produces fruits of outstanding skin finish and very well flavor balance.

## INTERSPECIFIC HYBRIDIZATION IN SWEET AND SOUR CHERRY BREEDING

M. Schuster<sup>1\*</sup>, C. Grafe<sup>1</sup>, E. Hoberg<sup>2</sup>, and W. Schütze<sup>2</sup>

<sup>1</sup>Institute for Breeding Research on Horticultural and Fruit Crops, Julius Kühn-Institut, Pillnitzer Platz 3a, D-02613 Dresden, Germany

<sup>2</sup>Institute for Ecological Chemistry, Plant Analysis and Stored Products Protection, Julius Kühn-Institut, Erwin-Baur-Straße 27, D-06484 Quedlinburg, Germany  
e-mail: mirko.schuster@jki.bund.de

Interspecific crosses are a valuable possibility to enlarge the gene pool in cherry for breeding and to insert new characteristics in cherry. The main goals in sweet and sour cherry breeding are the fruit quality, the resistance to biotic and abiotic stress and a stable fruit set. Collection of seven wild cherries were evaluated to resistance of leaf spot, *Blumeriella jaapii*, and the anthocyanin content. The diploid species *P. canescens* for sweet cherry and the tetraploid species *P. maackii* for the sour cherry were used as donors for resistance to leaf spot in different cross combinations. Currently BC<sub>1</sub> progenies were evaluated to study the inheritance of leaf spot resistance. The composition and the content of anthocyanins showed large differences in the studied species. The wild species *P. maackii* has the largest spectrum of anthocyanins. New anthocyanin compound have been found in F<sub>1</sub> hybrids of *P. cerasus* × *P. maackii*. Future studies should investigate the importance of the different anthocyanin compounds for the fruit and juice colour and the colour stability.

## TRENDS OF APPLE BREEDING PROGRAMS IN KOREA

**D. Kim<sup>1\*</sup>, S.-I. Kwon<sup>2</sup>, M.-J. Kim<sup>2</sup>, S. Heo<sup>2,3</sup>, J.H. Hwang<sup>2</sup>, H. Shin<sup>1</sup>, and Y.-U. Shin<sup>2</sup>**

<sup>1</sup>Department of Horticultural Science, Chungbuk National University, Cheongju 361-763, Korea

<sup>2</sup>National Institute of Horticultural & Herbal Science, Suwon 440-760, Korea

<sup>3</sup>Healthy Family Support Center, National Plant Quarantine Service, Seoul 121-886, Korea

In Korea, the ancient native cultivars were known originated from *Malus asiatica* Nakai and *M. micromalus* Makino, had been cultivated until 19 century. The apple breeding programs through hybridization have progressed mainly at the National Institute of Horticultural & Herbal Science (NIHHS) since 1954. Korean apple breeding programs could be divided into 3 periods by the specific breeding goals. Apple breeding program targeted to develop climatic adaptable cultivars in 1st period till early 1970s. Well coloration against 'Fuji' and reduction of pre-harvest drop against 'Tsugaru' were main goals in 2nd period. Main results in early periods until 1978 might be extending of various cross combinations and strengthening of techniques for future systematic apple breeding though any Korean cultivar could not release until that time. Six Korean apple cultivars, 'Hongro' (1988), 'Hwahong' (1992), 'Chukwang' (1992), 'Kamhong' (1992), 'Seokwang' (1995), 'Saenara' (1997) had released from the seedlings crossed in 3rd period since 1978. Especially, growing area of 'Hongro' apple has increased very rapidly for last 15 years from 0.06% in 1992 up to 10.6% in 2007. As results of breeding efforts to develop high quality in each harvesting season, whole of 20 cultivars developed by hybridization except point mutants are including 'Sunhong' (2000), 'Seohong' (2004), 'Hongeum' (2004), 'Summer Dream' (2005), 'Manbok' (2006), 'Hongso' (2006), 'Hongan' (2006), 'Ryehong' (2007), 'Picnic' (2008), 'Hwayoung' (2008), 'Danhong' (2008), 'Green Ball' (2008), 'Hwangok' (2009), 'Aisarang' (2009), 'Arisoo' (2010), 'Summer King' (2010). Over the 10,000 seedlings are planting in NIHHS in each year to adapt more complex objectives of Korean apple breeding programs which were more extended and specific to develop environment friendly, labor saving, and functional compound containing apple. Approaches for practical use of the biotechnology are also actively progressing to increase apple breeding efficiency through the molecular marker development and the transformation.

**Oral session**

**MANAGEMENT AND RESEARCH ON GENETIC  
RESOURCES**



## A CATALOG OF MOLECULAR DIVERSITY OF PRUNUS GERMPLASM GATHERED FROM ALIGNING NGS READS TO THE PEACH REFERENCE SEQUENCE: BIOINFORMATIC APPROACHES AND CHALLENGES

S. Scalabrin<sup>1</sup>, A. Policriti<sup>1,3</sup>, F. Nadalin<sup>1,3</sup>, S. Pinosio<sup>1</sup>, F. Cattonaro<sup>1</sup>, E. Vendramin<sup>4</sup>, V. Aramini<sup>4</sup>, I. Verde<sup>4</sup>, D. Bassi<sup>5</sup>, R. Pirona<sup>6</sup>, L. Rossini<sup>5,6</sup>, R. Testolin<sup>1,2\*</sup>, and M. Morgante<sup>1,2</sup>

<sup>1</sup>Istituto di Genomica Applicata, PST ‘L. Danieli’, Via J. Linussio 51, 33100 Udine, Italy

<sup>2</sup>Dipartimento di Scienze Agrarie e Ambientali, Via delle Scienze 208, 33100 Udine, Italy

<sup>3</sup>Dipartimento di Matematica e Informatica, Via delle Scienze 208, 33100 Udine, Italy

<sup>4</sup>CRA - Centro di Ricerca per la Frutticoltura, Via di Fioranello 52, 00134 Roma, Italy

<sup>5</sup>Dipartimento di Produzione vegetale, Via Celoria 2, 20133 Milano, Italy

<sup>6</sup>Fondazione ‘Parco Tecnologico Padano’, Via Einstein , Loc. Cascina Codazza, 26900 Lodi, Italy

Genome analysis based on next generation sequencing (NGS) technologies provides a powerful approach for surveying molecular diversity among individuals, which in turn can generate tools for linkage mapping, association mapping, gene cloning, molecular breeding, population genetics, germplasm management, crop systematics and evolution. ‘De novo’ assembly of short reads is challenging as far as the size and complexity of genomes increases. A reference genome correctly assembled and annotated can help solving most of the problems, although several structural variants such as the movement of transposable elements, large insertions/deletions, segmental duplications and other genomic features are still challenging algorithms and automatic procedures. We sequenced 16 *Prunus* accessions, that include 14 peach cultivars and species, one almond and one apricot varieties, using the NGS Illumina platform. We produced 64 to 109 bp long single reads as well as paired ends from approx. 300 - 500 bp long fragments. The coverage varied from approx. 16 to 75 genome equivalents. Individual genomes were aligned using the doubled haploid peach cultivar ‘Lovell’ reference sequence, recently released by the International Peach Genome Initiative (IPGI) (<http://www.rosaceae.org/peach/genome>). In this paper we present a catalog of molecular variants that can be mined, namely SNPs (Single Nucleotide Polymorphisms), DIPs (Deletion/Insertion Polymorphisms), larger structural variations, which include movement of transposable elements, the so called copy-number variations, segmental duplications and other. Some of these variants, such as SNPs, are easily detected and many commercial and open-access software can perform the search. Others variants, such as the large structural variations, have still analytical approaches to be implemented or improved. For most variants, the possible methodological approaches are discussed and, when available, preliminary results are reported.

## DEVELOPMENT, CHARACTERIZATION AND USE OF MICROSATELLITE MARKERS FOR GERMPLASM ANALYSIS IN DATE PALM (*Phoenix dactylifera* L.)

H. Arabnezhad<sup>1</sup>, M. Bahar<sup>1\*</sup>, H. Reza Mohammadi<sup>1</sup>, and M. Latifian<sup>2</sup>

<sup>1</sup>Department of Agricultural Biotechnology, College of Agriculture, Isfahan University of Technology, Isfahan 84156, Iran

<sup>2</sup>Date Palm and Tropical Fruits Research Institute of Iran, P.O.Box 61355-16, Ahwaz, Iran

Date palm (*Phoenix dactylifera* L.) is a preferred tropical fruit tree which geographically extends through South West Asia, Middle East and North Africa. However, there is little information available concerning genetic relatedness within and between date palm genotypes grown in different geographical regions. The present study was undertaken to assess genetic relationships among commercially important genotypes of date palm by using simple sequence repeat (SSR) markers. Two SSR-enriched genomic libraries including repeat motifs (AG)<sub>n</sub> and (AAG)<sub>n</sub> were constructed in date palm. PCR colony screening technique was used to determine SSR-containing clones. DNA sequences were obtained for 47 positive clones and 25 primer pairs were designed, of which 22 pairs were able to detect polymorphism in 16 date palm cultivars from Iran, Iraq and Africa. The selected SSR primers amplified a total of 106 alleles with an average of 4.82 alleles per locus among the cultivars and the average values of He and PIC were 0.719 and 0.668, respectively. Neighbor-Joining cluster analysis based on Nei's genetic distance divided date palm accessions into three major clusters in agreement with their geographical origin. Cluster analysis significantly distinguished African cultivars from Iranian and Iraqi ones, suggesting that the domestication of African date palms have followed a different route than those grown in the Middle-East, an assumption which is supported by Mantel test and Bayesian analysis. The set of date palm SSR loci developed in the present study could be an informative marker system for geographic partitioning and genotyping of date palm germplasm collections worldwide.



## TESTING DIFFERENT APPROACHES TO CONSTRUCT OLIVE (*Olea europaea* L.) CORE SUBSET SUITABLE FOR ASSOCIATION GENETIC STUDIES

A. Elbakkali<sup>1,2,3,4\*</sup>, H. Haouane<sup>1,2</sup>, P. Van Damme<sup>4</sup>, and B. Khadari<sup>1,5</sup>

<sup>1</sup>INRA, UMR 1334 Amélioration Génétique et Adaptation des Plantes (AGAP), F-34398 Montpellier, France

<sup>2</sup>Montpellier SupAgro, UMR 1334 AGAP, F-34398 Montpellier, France

<sup>3</sup>INRA, CRRA-Meknès, UR Amélioration des Plantes et Conservation des Ressources Phytogénétiques (APCRPG), B.P. 578, Meknès, Morocco

<sup>4</sup>University of Ghent, Faculty of Bioscience Engineering, Coupure links 653 B-9000, Ghent-Belgium

<sup>5</sup>CBNMED, UMR 1334 AGAP, F-34398 Montpellier, France

Evaluation of the genetic diversity is of great interest for management and crop breeding program. Management can be efficient when the evaluation is focused on a subset of accessions with reduced size that represents the variability observed in the whole germplasm collection. Most of core sets have been developed for many seed crops by using different approaches and sampling size to select entries on the basis of genetic and phenotypic data, but the corresponding studies in perennial crops are very fewest. Here, we constructed a core subset for olive (*Olea europaea* L.) using both Simple Sequence Repeats (SSRs) markers and agronomic criteria, by testing diverse sampling approaches including stratified and non-stratified methods. Twelve SSRs markers are used to construct a core subset from an initial collection of 505 unique genotypes issued from 14 Mediterranean countries. Among all the allocation methods, we showed that the sample size of 12.5% was most suitable in capturing all the observed alleles using M-strategy approach. Based on both SSRs markers and agronomic data, we established a core set including the main Mediterranean varieties and displaying the highest genetic and phenotypic variability. The model-based program STRUCTURE and Principal Component analysis (PCA) were used to study the genetic structure of the established core subset. The results indicate no obvious genetic structure into the core subset. Our results gave an efficient basis as a first step for olive association mapping. The decay of linkage disequilibrium according to the genetic distance will be assessed on this core subset. It could be further evaluated for traits of agronomic interest, leading to association between the allelic variation and the phenotypic variability.



**Oral session**

**FRUIT CROP PHYSIOLOGY - REPRODUCTIVE  
BIOLOGY**



## EVALUATION OF CITRUS SEED TOLERANCE TO DESICCATION AND LIQUID NITROGEN STORAGE: TOWARDS CITRUS GERMPLASM CRYOPRESERVATION

F. Luro<sup>1\*</sup>, I. Tur<sup>1</sup>, and S. Dussert<sup>2</sup>

<sup>1</sup>Unité de Recherche GEQA 1103, station INRA 20230 San Giuliano France

<sup>2</sup>IRD, 911 Avenue d'Agropolis, BP 64501, F-34394, Montpellier, France

Preservation of plant genetic resources represents a big challenge for *ex situ* germplasm curators. For citrus, as well as many other fruit species, genetic resources are preserved in field collections. However, this method of conservation does not ensure the long time preservation of genetic resources because trees remain exposed to climatic changes and pest pressures. Moreover, it is land consuming and expensive. One promising alternative could be the development of seed banks since many citrus varieties produce polyembryonic seeds which allow clonal propagation. Unfortunately citrus seeds may display a non-orthodox seed storage behaviour, excluding then standard seed banking approaches for long-term *ex situ* conservation. Cryopreservation represents an alternative strategy for long-term storage of non-orthodox seeds. In the present work, we investigated seed tolerance to desiccation and cryopreservation for a very large set of varieties representing 15 species from *Citrus* and related genera. In contrast with the initial assumption supporting that tropical and subtropical plants are more sensitive to desiccation than plants from other origins, *Citrus* species that originate from southern countries (India-Indonesia-south China) were more tolerant to desiccation than *Poncirus* and *Fortunella* which originate from northern area of China. Seeds of these two last genera were highly sensitive to desiccation. Differences in seed behaviour were also observed between the three genera regarding seed water loss kinetics and water sorption characteristics. All species genetically related to citron, such as lime and lemon, were highly tolerant to desiccation. Pummelo could be considered as intermediate, while contrasted behaviours were observed within the mandarin group, Sunki being the most tolerant variety and Cleopatra the most sensitive. According to their genetic origin, grapefruits, oranges and sour oranges showed a similar behaviour to that of their ancestors. The optimal hydration status for liquid nitrogen exposure was usually achieved by equilibration drying at 75-80% relative humidity. Moreover, heritability of seed desiccation tolerance was demonstrated by investigating hybrids from crosses between sensitive and intermediate parental varieties. This study provides important applied perspectives for citrus germplasm cryopreservation and future genetic studies in terms of QTL analysis of tolerance to desiccation.

## QUANTITATIVE AND QUALITATIVE EXPRESSION OF SELF - COMPATIBILITY IN ALMOND

**R. Socias i Company, À. Fernández i Martí, O. Kodad, and J.M. Alonso**

Unidad de Fruticultura, Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA), Av. Montañana 930, 50059 Zaragoza, Spain

The evidence of almond self-pollinations through near a century has shown a high variability of fruit sets, ranging from nil, indicating self-incompatibility, to very high, reaching the level of a normal crop and confirming self-compatibility in these genotypes. All intermediate levels of fruit set were already found in the first study identifying self-compatibility by Almeida. However, most attention has been directed towards the qualitative presence of the  $S_f$  allele, especially after that this allele could be easily identified by specific primers after PCR in the lab, and not to this quantitative expression of self-compatibility, as measured in field trials. Recent findings have evidenced that the presence of the  $S_f$  gene is not the exclusive source of self-compatibility in almond, arising as a consequence the question of modifier genes affecting its expression and reviving the previous suggestion that almond may be a self-incompatible species with a genetic background of pseudo-self-compatibility, as indicated by the small self set observed in some cultivars. Over this background, only one  $S_f$  allele could break the self-incompatible system, but probably interacting with this background of pseudo-self-compatibility where several minor genes could act to produce a quantitative expression of self-compatibility in almond.

## STIGMATIC RECEPTIVITY LIMITS FRUIT SET IN ALMOND UNDER WARM CLIMATES

O. Kodad<sup>1</sup>, Z. Messaoudi<sup>1</sup>, A. Mamouni<sup>2</sup>, M. Lahlou<sup>2</sup>, and R. Socias i Company<sup>3\*</sup>

<sup>1</sup>Département d'Arboriculture, École Nationale d'Agriculture de Meknès, BP S/40, Morocco

<sup>2</sup>Unité de Recherche, Amélioration des Plantes et Conservation des Ressources Phytogénétiques, INRA, Meknès, Morocco

<sup>3</sup>Unidad de Fruticultura, CITA de Aragón, Av. Montañana 930, 50059 Zaragoza, Spain

The cause for the erratic yields of two almond cultivars has been searched in order to recommend possible solutions. The lack of sufficient bloom overlap between 'Marcona' and 'Fournat de Brézenaud' in many years may be one of the reasons of this erratic behaviour, but not the only one, as the same behaviour may be observed in two simultaneously blooming cultivars, 'Ferragnès' and 'Ferraduel'. The relatively high temperatures observed during the blooming period significantly reduced the stigma receptivity of these cultivars and, as a consequence, their effective pollination period, showing that stigmatic receptivity may be a limiting factor for fruit set in 'Marcona' and 'Ferragnès' and their subsequent yield reduction under warm conditions. Thus, in the present context of global warming, the search for cultivars tolerant to heat stress during flowering will acquire a special interest, as well as the combination of cultivars with the same chilling and heat requirements in order to ensure their simultaneous bloom.





**Oral session**

**FRUIT CROP PHYSIOLOGY - VEGETATIVE  
PROPERTIES**



## **ETHREL APPLICATION SHORTENS THE JUVENILE PHASE OF APPLE SEEDLINGS**

**E. Żurawicz\* and M. Lewandowski**

Fruit Breeding Department, Research Institute of Horticulture, Konstytucji 3 Maja 1/3,  
96-100 Skierniewice, Poland  
e-mail: Edward.Zurawicz@insad.pl

A study was conducted between 1998 and 2007. Its aim was to work out a method that allows the shortening of the juvenile phase of apple seedlings in conventional apple breeding programs. The investigation was based on two separate experiments, each conducted first in high plastic tunnel and then in the field. In both experiments in the first two years, the seedlings were grown on their own roots in plastic containers (cylinders) placed in the high plastic tunnels. At the end of the second year, shoot tips from the seedlings were collected and grafted onto dwarf growing rootstock M.9 (winter hand grafting). At the end of March, the grafts were planted in plastic containers and were placed in the same high plastic tunnel, where they were cultivated until the middle of August, when they were planted out in the selection field. In the first experiment seedlings, belonging to two families obtained from the cross between 'Ligol' × 'Delbard Jubile' were investigated. In the second experiment also two seedling populations were tested, but these originated from a cross made between 'Free Redstar' × 'Melodie' and 'Free Redstar' × 'Coop 38'. In the first experiment, nine experimental treatments were studied. They involved two growing media (peat substrate and mixture of peat substrate with compost soil 1:1), two mineral fertilizers (Osmocote Plus and Polish fertilizer Azofoska) and two bioregulators (Gibrescol 10 MG containing GA<sub>3</sub> and Ethrel). In the second experiment additionally compost soil was included. In both experiments each treatment comprised of 32 seedlings divided into 4 replications with 8 plants in each replicate. It was found that Ethrel applied on young apple seedlings grown in high plastic tunnel (in the first year of cultivation) shortened the juvenile phase of these seedlings grown on M.9 rootstock in the selection field. In the first year after planting in the selection field (third year after seed sowing) 25% of the seedlings produced flowers, and in the second year all of them flowered and produced fruits and it was possible to make the first fruit evaluation.

## **A GENETIC STUDY OF THE VEGETATIVE AND REPRODUCTIVE DEVELOPMENT IN A F1 PROGENY ‘OLIVIÈRE’ X ‘ARBEQUINA’: TOWARD A BETTER UNDERSTANDING OF OLIVE TREE ONTOGENY**

**I. Ben Sadok<sup>1,2\*</sup>, G. Garcia<sup>1</sup>, N. Moutier<sup>1</sup>, F. Dosba<sup>2</sup>, B. Khadari<sup>1</sup>, and E. Costes<sup>1</sup>**

<sup>1</sup>INRA, UMR AGAP 1334 – Amélioration Génétique et Adaptation des Plantes méditerranéennes et tropicales, Campus Cirad- TA-A-108/03, Av. Agropolis, 34398 Montpellier Cedex 5, France

<sup>2</sup>Montpellier SupAgro, UMR AGAP 1334 – Amélioration Génétique et Adaptation des Plantes méditerranéennes et tropicales, Campus Cirad- TA-A-108/03, Av. Agropolis, 34398 Montpellier Cedex 5, France

The agronomic performance of fruit trees has been shown to be significantly influenced by trees internal organization and volume. Trees architecture has a major impact on their adaptability to cultivation systems, but also yields and fruit quality. Thus, there is an interest for introducing architectural traits in breeding programs for selecting new varieties with a regular bearing and for reducing training and environmental costs. However, an interaction between tree ontogeny and genetic factors is expected that needs to be clarified beforehand. In the present study, we aimed at investigating the genetic determinism of architectural traits in the olive tree. A F1 hybrid population was created between two genotypes with contrasted architecture traits, ‘Olivière’ and ‘Arbequina’. A phenotyping methodology was applied to decompose tree architecture in a number of quantitative traits, which represent its complexity over time. Traits related to growth, branching and fruiting behaviour, measured during the first 5 years of tree growth were analysed taking into account the influence of the year of growth, branching order and genotype. The genetic factor significance was variable with the position of growth units within the tree, and the year of growth. Heritable traits were though identified. Geometrical variables showed medium to high broad sense heritability values. The highest heritability values were found for the basal diameter with  $H^2$  value greater than 0.7. Flowering and fruiting variables showed high heritability values reaching 0.78 for fruit set percentage. These results were interpreted with respect to tree ontogeny and led us to propose phenotyping strategy adapted to olive tree.

## EXPRESSION OF THE 4-COUMARATE: CoA LIGASE GENE FAMILY IN COMPATIBLES AND INCOMPATIBLES *Prunus* GRAFTS

I.S. Pereira<sup>1</sup>, J.C. Fachinello<sup>1</sup>, L.E.C. Antunes<sup>2</sup>, P. Errea<sup>3</sup>, R.S. Messias<sup>2</sup>, and A. Pina<sup>3\*</sup>

<sup>1</sup>Departamento de Fruticultura, Universidade Federal de Pelotas (UFPel), Brazil

<sup>2</sup>Departamento de Fitotecnia e Fruticultura, Embrapa Clima Temperado (CPACT), Brazil

<sup>3</sup>Unidad de Fruticultura, Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA), Zaragoza. Spain

Graft incompatibility is actually an important problem for development of new *Prunus* rootstocks. Recently, several authors have reported that the phenylpropanoid metabolism is related with this phenomenon. In plants, this pathway is constituted for three key enzymes, phenylalanine ammonia-lyase (PAL), cinnamate 4-hydroxylase (C4H), and 4-coumarate:CoA ligase (4CL). The 4CL family is especially important in this pathway, as they catalyze the conversion of the hydroxylated cinnamic acids to their respected thioesters. These cinnamyl CoA esters are used for biosynthesis of several phenolic compounds required for various physiological functions and adaptation to environmental perturbations. The aim of the present work was to analyze the gene expression patterns of the 4-Coumarate:CoA genes in compatibles and incompatibles graft combinations two years after grafting. The relationship of *Prunus* 4CLs with other plants was also studied. To perform this study different graft combinations were evaluated: three rootstocks cultivars, 'Capdeboscq' (*Prunus persica* L. Batsch), 'Tsukuba 1' (*P. persica* L. Batsch) and 'Umezeiro' (*P. mume* Sieb. et Zucc.) grafted into a peach cultivar, 'Chimarrita' (*P. persica* L. Batsch). Transcript abundance was analysed by qRT-PCR using gene-specific primers designed to amplify the three 4CL genes identified in the assembled peach genome. The results showed that the three 4CL transcripts are expressed in all combinations examined, although the quantitative levels of 4CL mRNA were higher in 'Umezeiro', the incompatible combination. 4CL3 gene was the most highly expressed in the incompatible combination; and it is also the most divergent isoform in the phylogenetic analysis. Based on these results, it seems to be clear the involvement of the phenylpropanoid pathway and especially 4CL enzymes in the incompatibility scion-stock interaction.



**Oral session**

**GENETIC AND GENOMIC STUDIES OF FRUIT  
QUALITY**





## GENETIC RELATIONSHIPS AMONG FRUIT QUALITY TRAITS IN RED-FLESHED APPLES

**R.K. Volz<sup>1\*</sup>, S. Kumar<sup>1</sup>, D. Chagné<sup>2</sup>, R. Espley<sup>3</sup>, and A.C. Allan<sup>3</sup>**

The New Zealand Institute of Plant & Food Research Ltd (Plant & Food Research)

<sup>1</sup>Hawke's Bay Research Centre, Private Bag 1401, Havelock North 4157, New Zealand

<sup>2</sup>Palmerston North Research Centre, Private Bag 11600, Palmerston North 4442, New Zealand

<sup>3</sup>Mt Albert Research Centre, Private Bag 92169, Auckland 1142, New Zealand

e-mail: Richard.Volz@plantandfood.co.nz

An understanding of the genetic architecture of important monogenic and polygenic traits is essential in optimizing parental and progeny selection strategies in plant breeding. Identification and estimation of genetic relationships among these traits constitutes an important part of this architecture. While such relationships for common fruit quality traits in apple have been published in the last decade, these relationships need to be continually assessed. This is particularly so where new genes are introgressed from the wider *Malus* germplasm pool. Plant & Food Research has a breeding strategy of discovering, developing and introgressing novel traits into apple breeding populations. Thus an understanding of genetic relationships is particularly important for the ultimate success of this strategy. Recently we have explored the relationships between the red flesh character and a number of traditional fruit quality traits as well as fruit phytochemical composition and expression of an internal browning disorder. These relationships were determined by estimating genetic correlations in breeding populations through classical quantitative genetics, genetic mapping using molecular markers and/or a functional genomics approach using genetically modified red-fleshed apples. This presentation will summarize some preliminary results of these studies.

## BIOCHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF FRUIT CROPS

**N.I. Savelyev<sup>1\*</sup>, V.N. Makarov<sup>1</sup>, A.N. Jushkov<sup>1</sup>, N.N. Savelyeva<sup>1</sup> N.V. Borzykh<sup>1</sup>, and M.J. Akimov<sup>2</sup>**

<sup>1</sup>All-Russian Research Institute for Genetics and Breeding of Fruit Plants, Tambov region, Michurinsk, Russia

<sup>2</sup>Management for Realization of the Programme of Michurinsk Development as Town of Science of Russian Federation Tambov region, Michurinsk, Russia  
e-mail: cglm@rambler.ru

One of the major components of balanced prophylaxis and natural source of antioxidants are fresh fruits and berries. The purpose of the research was to estimate the content of vitamin C, P-active catechins, pectin, arbutin, anthocyanins, chlorogenic acid and other active compounds in cultivars of the genetical collection of pome fruits – apple (*Malus × domestica*), pear (*Pyrus communis* L.), black currant (*Ribes nigrum* L.), strawberry (*Fragaria × ananassa*) and raspberry (*Rubus idaeus* L.) The total antioxidant activity was also assessed. The genotypes most valuable from the biochemical point of view were selected. ‘Uspenskoye’, ‘Martovskoye’, ‘Roskoshnoye’ apples combine in one genotype a high content of vitamin C (more than 20 mg per 100 g) and of P-active catechins (more than 250 mg per 100 g). The highest total antioxidant activity was noted in ‘Stroyevskoye’, ‘Start’, ‘Martovskoye’ and ‘Bylina’. Pear cultivars ‘Pamyati Yakovleva’, ‘Nezhnost’, were characterized by a higher content of P-active catechins, arbutin, chlorogenic acid a higher total antioxidant activity. The high content of ascorbic acid was found in ‘Krupnoplodnaya’, ‘Amur’ and ‘Orlovskaya serenada’ black currants (more than 200 per 100 g), in ‘Privlekatelnaya’, ‘Urozhainaya CGL’ and ‘Maryshka’ strawberries (56,8 - 72,3 mg/100g) as well as in ‘Gerakl’ and ‘Zolotaya osen’ raspberries (about 37 mg per 100g). The vitamin P content was the highest in the black currant cultivar Orlovskaya serenada (904 mg per 100 g) The highest concentration of anthocyanins was found in the ‘Sevchanka’ and ‘Muravushka’ black currants (325.6 - 369.6 per 100 g), Eurasia’ and ‘Gerakl’ raspberries (149.6 and 198.0 per 100 g) and in ‘Privlekatelnaya’ and ‘Feiyerverk’ strawberries (101.2 - 118.8 per 100 g). The highest total antioxidant activity was noted in black currant cultivars (413.7 mg per 100 g on the average). This indicator was rather high (369.1 mg per 100 g) also in raspberries; less antioxidants were found in strawberries (236.5 mg per 100 g). The selected forms have been recommended as complex sources of genes in breeding for higher content of biochemical substances in fruits and thus obtaining natural high vitamin products.

## **CLONING AND CHARACTERIZATION OF APPLE LIPOXYGENASE (LOX) GENES PUTATIVELY INVOLVED IN THE FORMATION OF FRUIT FLAVOUR VOLATILES**

**J. Vogt<sup>1\*</sup>, D. Schiller<sup>2</sup>, W. Schwab<sup>2</sup>, and F. Dunemann<sup>1</sup>**

<sup>1</sup>Julius Kühn-Institut (JKI), Bundesforschungsinstitut für Kulturpflanzen, Institut für Züchtungsforschung an gartenbaulichen Kulturen und Obst, 01326 Dresden, Germany

<sup>2</sup>Biotechnologie der Naturstoffe, Technische Universität München, 85354 Freising, Germany

Even if apple aroma is recognized as an important quality attribute worldwide and one of the main goals in breeding programs, this trait can still be considered as a random result of traditional breeding, due to its complex genetic control and the large-scale methodology required for its biochemical characterization (metabolomic phenotyping). Important progress in improving fruit quality of resistant apple cultivars could be achieved by molecular breeding based on a new generation of functional markers developed for key genes of the most important biosynthetic pathways. Lipoxygenases (LOX) catalyze the addition of molecular oxygen to polyunsaturated fatty acids to produce their respective hydroperoxides and have many different putative physiological functions in higher plants. The LOX reaction provides also important substrates for the formation of both aroma-active C6-aldehydes as well as hexyl and hexenyl esters that decisively shape the aroma pattern of ripe and stored apple fruits. The objective of the presented research is to identify and characterize LOX gene family members by co-localization with known volatile QTLs as well as by temporal and spatial expression patterns determined in different phases of fruit development. First results of bioinformatic sequence studies as well as from PCR-based full-length cloning of LOX genes will be presented, including a phylogenetic tree based on alignments of cloned LOX genes aligned to genes determined by gene mining in the whole “Golden Delicious” genome sequence. Based on this tree and on sequence similarity we were able to classify LOX genes in apple in different groups and could confirm the genes of the apple genome draft sequence as well as published LOX-encoding genes from apple EST databases. Presently we want to find out by RT-PCR-based expression analyses and biochemical protein characterization, which LOX genes are crucial for the production of precursors for key fruit flavor volatiles.

## **DIVERSITY ANALYSIS OF CITRUS FRUIT PULP ACIDITY AND SWEETNESS: TOWARD TO UNDERSTAND THE GENETIC CONTROL OF THE FRUIT QUALITY PARAMETERS**

**F. Luro\*, J. Gatto, G. Costantino, and O. Pailly**

Unité de Recherche 1103 Génétique et Ecophysiologie de la Qualité des Agrumes (GEQA), station de recherche INRA, 20230 San Giuliano, Corse, France

Acidity level is the main criteria of quality and physiological maturity evaluation for citrus fruit, especially for mandarins and oranges. The constituents of the citrus fruit pulp acidity are organic acids and among them citric acid is the major one. Their amounts depend on species, variety, rootstock, environmental factors such as temperature and rainfall, and the acidity level varies during fruit development and maturation. Acidity metabolism is linked to sugar metabolism. To have a better control of fruit harvest, it is necessary to understand the genetic control and the parameters influencing the variation of acidity and sweetness. 87-varieties belonging to the 8 major *Citrus* species grown under the same environmental and cultivation conditions were selected to have a good representation of the *Citrus* diversity. The composition of juice in primary metabolic components was analysed by HPLC (High Performance Liquid Chromatography). The sequence polymorphism of 9 candidate genes encoding for key enzymes of sugars and organic acids metabolic pathways was investigated by SSCP (Single Strand Conformation Polymorphism) and confirmed by sequencing. Whatever the biochemical or molecular analyse, the observed structure of the *Citrus* diversity was organized around three groups corresponding to the ancestral species (mandarin, pummelo and citron). As expected, the secondary species were closely related to their putative species genitors. We could display that the biochemical diversity was strongly correlated to molecular SSCP diversity without having any certainty on the cause and effect relationship. Mandarin group can be characterized by specific characters such as high amount of succinic acid in the pulp and a very important acidity decrease during fruit maturation. A higher diversity has been observed with SSCP and sequencing technologies than with other molecular markers. Few varieties of sweet orange and grapefruit which evolved only by mutations could be distinguished by single nucleotide polymorphism. These results offer the opportunity to develop future QTL mapping approach for understanding the genetic control of citrus pulp acidity regulation.

## GENETIC STUDY ON FRUIT STORAGE LIFE IN DISCONNECTED FACTORIAL MATING POPULATION OF *Actinidia chinensis* (KIWIFRUIT)

**C. Cheng\* and S. Day**

The New Zealand Institute for Plant & Food Research Limited, Mount Albert Research Centre, Private Bag 92 169, Auckland, New Zealand  
e-mail: Canhong.cheng@plantandfood.co.nz

The purpose of this study was to examine inheritance of storage life (estimated by fruit firmness (FF)) in a population of *Actinidia chinensis*, to select parents for improving storage life of kiwifruit. A total of 708 female vines were sampled in 2010, from a disconnected mating factorial, with 2 x 6 and 2 x 2 sub-factorials and a total of 16 full-sib families. To maximize accuracy of the prediction of breeding values, best linear unbiased prediction (BLUP) was performed with pedigree information, using ASReml-R. The coefficients of coancestry for the 16 full-sib families were 0, whereas the coefficients for disconnected families (not crossed) were from 0.188 to 0.625. Seedlings were sequentially harvested from April to June as their soluble solids concentration (SSC) reached 10%. FF was measured after 56 - 64 days storage at 1.5°C. Narrow-sense heritability and BLUPs of FF for each vine at 120 days in storage were calculated, using mixed model in analysis of covariance in ASReml-R, with 'days in storage' as the covariate. General combining ability (GCA) and specific combining ability (SCA) of BLUPs were tested. There was significant GCA ( $p < 0.01$ ) for both female and male parents. Parents useful for improving storage life were identified. Heritability was relatively high (0.62), which indicated that fruit storage life could be improved through selection. The response to selection of FF was 0.32 kgf at 1% selection pressure. FF also had a positive genetic correlation with dry matter (DM) and SSC. The relative selection efficiencies of direct selection for FF using DM and SSC were 0.32 and 0.33 kgf, suggesting that selection using DM/SSC could be a convenient and efficient alternative to direct selection for improving storage life. Our results showed that the disconnected factorial design using BLUPs can be an efficient and economic procedure for identifying superior parents.

## **PRELIMINARY RESULTS FOR INHERITANCE OF BLUSH IN PEAR CROSS COMBINATIONS**

**J.P. Human**

ARC Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch, 7599, Republic of South Africa

e-mail: [humant@arc.agric.za](mailto:humant@arc.agric.za)

In South Africa, breeding of a range of blush cultivars of pear (*Pyrus communis*) is of high priority as this pear type is still regarded on overseas markets as novel and it fetches a price premium over normal green/yellow cultivars. Early successes of the ARC in breeding blush cultivars such as ‘Rosemary’ (released 1990) and ‘Flamingo’ (released 1993), and the extension of the range with the more recent cultivar ‘Cheeky’, released in 2009, created an opportunity for South African pear producers to capitalise on the high prices realised on export markets with these cultivars. However, blush appears to be a quantitative trait and the genetics associated with its inheritance are still not fully understood. This presentation will cover preliminary results recorded in several families and a discussion, based on one season’s data only, and an outline will be presented of further prospects such as the utilization of microsatellite markers to generate a genetic map indicating the location of genes/alleles associated with the blush trait.

## ASSESSMENT OF *Mal d 1* GENES DIVERSITY IN CONTEMPORARY AND HISTORICAL POLISH APPLE CULTIVARS

**K.L. Bokszczanin<sup>1</sup>, A.A. Przybyla<sup>2</sup>, and M. Filipecki<sup>1</sup>**

Warsaw University of Life Sciences – SGGW, 159 Nowoursynowska Str., 02-776, Warsaw, Poland

<sup>1</sup>Department of Plant Genetics, Breeding and Biotechnology

<sup>2</sup>Department of Pomology

*Mal d 1* is a major apple allergen causing food allergic symptoms of the oral allergy syndrome (OAS) resulted from IgE-mediated cross-reactivity between Bet v 1, the major birch allergen, and Mal d 1. The *Mal d 1* gene family is known to have at least 7 intron-containing and 11 intronless members that have been mapped in clusters on three linkage groups. Since it is known, that compositions of particular *Mal d 1* genes are associated with the differences in allergenicity, we assessed the allelic diversity of *Mal d 1.01*, *Mal d 1.02*, *Mal d 1.04*, *Mal d 1.06 A-C* genes by sequencing. Among a set of apple cultivars 33 new alleles were identified.





**Oral session**

**BIOTECHNOLOGY AND MOLECULAR BREEDING**



## CISGENESIS FITS IN THE TOOLKIT OF A MODERN FRUIT BREEDER

**T. den Nijs\*, H. Schouten, and F. Krens**

Wageningen UR Plant Breeding, P.O. Box 386, 6700 AJ Wageningen, The Netherlands  
e-mail: ton.dennijs@wur.nl

Cisgenesis is the one-step introgression of a relevant gene from a crossable species into an existing cultivar, equivalent to five or six generations of backcrosses in a conventional breeding program. The enormous time gain renders this new biotechnological technique extremely interesting for breeders wishing to improve outstanding cultivars with specific extra characters. It avoids the linkage drag associated with wide crosses and leaves the genetic make up of the recipient cultivar intact. No extra marker genes are left after the transformation procedure. However, technically a cisgenic cultivar must be labelled in Europe as a genetically modified organism (gmo), but there are compelling reasons to exempt these cultivars from the cumbersome and expensive gmo regulations. This exemption is a "*conditio sine qua non*" for applying this technique by SMEs which is the rule in the fruit breeding business. In close collaboration with Inova Fruit, ETH Zurich, Switzerland, and Plant and Food Research, New Zealand, Wageningen UR Plant Breeding has created the first cisgenic trees of cv Gala with the *Vf* gene for scab resistance or the *Myb10* gene for red fruit flesh. Trees will be planted in an experimental orchard at Wageningen this fall.

## HEAT-SHOCK REGULATED EXCISION OF THE *NPTII* MARKER GENE IN TRANSGENIC APPLE (*Malus × domestica* BORKH.)

K. Herzog<sup>1,2</sup>, J. Würdig<sup>1\*</sup>, H. Flachowsky<sup>1</sup>, H.B. Deising<sup>3</sup>, and M.-V. Hanke<sup>1</sup>

<sup>1</sup>Julius Kühn-Institut (JKI), Bundesforschungsinstitut für Kulturpflanzen, Institut für Züchtungsforschung an gartenbaulichen Kulturen und Obst, 01326 Dresden, Germany

<sup>2</sup>Julius Kühn-Institut (JKI), Bundesforschungsinstitut für Kulturpflanzen, Institut für Rebenzüchtung, 76833 Siebeldingen, Germany (present address)

<sup>3</sup>Martin-Luther-Universität Halle-Wittenberg, Institut für Pflanzenzüchtung und Pflanzenschutz, Professur Phytopathologie und Pflanzenschutz, 06120 Halle (Saale), Germany

Breeding of apple *Malus × domestica* Borkh. is time consuming and expensive. Genetic engineering seems to be useful to overcome the limitations of classical breeding. Selected genes/traits are introduced into the genome of existing cultivars via *Agrobacterium*-mediated plant transformation. The final product will be a cultivar with improved traits which is already established on the market. Especially the transfer of genes from crossable species into existing cultivars followed by the elimination of all unwanted DNA sequences (e.g. selectable marker gene) from the final product is expected to be more acceptable for consumers than classical transgenic plants. In the present study a method to develop marker-free genetically modified (gm) plants in apple was established. The method is based on a site-specific excision of the *nptII* marker gene, which is mediated by a heat-shock inducible expression of the FLP recombinase driven by the soybean *GmHsp17.5-E* promoter. A monitoring vector contain in the *nptII* and the *flp* genes in a box flanked by two direct repeated FRT recognition sites was developed. The FRT-flanked box separates the *gusA* reporter gene from the *CaMV35S* promoter. GUS expression is only expected after excision of the FRT-flanked box. Using this vector, nine gm lines were produced and investigated by PCR, RT-PCR and Southern hybridization. Different temperature regimes were tested and after exposure of gm shoots to 42°C for 4 hours a high number of recombination events were detectable. Subsequent shoot regeneration from leaf explants was necessary to obtain gm apple plants completely free of *nptII*. To avoid the additional shoot regeneration cycle, we tested three different induction methods on leaves or leaf explants, respectively. Furthermore, first results of additional experiments aimed on improving the system will be presented.

## INTROGRESSION OF A MAJOR QUANTITATIVE TRAIT LOCUS FOR FIRE BLIGHT RESISTANCE USING EARLY FLOWERING TRANSGENIC APPLE TREES

P.-M. Le Roux<sup>1,3\*</sup>, H. Flachowsky<sup>2</sup>, M.-V. Hanke<sup>2</sup>, C. Gessler<sup>3</sup>, and A. Patocchi<sup>1</sup>

<sup>1</sup>Plant Protection and Extension Fruits and Vegetables, AgroscopeChangins-Wädenswil ACW, Schloss, 8820, Wädenswil, Switzerland

<sup>2</sup>Julius Kühn-Institute, Federal Research Centre for Cultivated Plants, Institute for Breeding Research on Horticultural and Fruit Crops, PillnitzerPlatz 3a, D-01326 Dresden, Germany

<sup>3</sup>Plant Pathology, Institute of Integrative Biology, ETH Zurich, Universitätstrasse 2, CH 8092 Zurich, Switzerland

e-mail: andrea.patocchi@acw.admin.ch

Fire blight (FB), caused by the bacterium *Erwinia amylovora*, is a major disease of the domesticated apple (*Malus × domestica* Borkh.). A recent epidemic of fire blight in Switzerland (2007) led to the destruction of 100 ha of apple trees, representing 30 million Swiss Francs of financial losses for the Swiss Confederation. Most apple cultivars are susceptible to the disease, therefore breeding for host resistance can be considered as an essential component of an integrated strategy to manage efficiently and durably fire blight. In this study, we report the first steps of the fast introgression of a major Quantitative Trait Locus (QTL) for fire blight resistance from the ornamental apple cultivar ‘Evereste’, using early flowering apple tree transformed with the *BpMADS4* gene (T1190 line). The integration site of the T-DNA carrying the *BpMADS4* gene was mapped on the linkage group (LG) 4 of T1190, meaning that the transgene and the QTL (mapped previously on LG12) are expected to segregate independently. Trees of the transgenic line T1190 were pollinated by ‘Evereste’ in early 2009. Fifty seven F1 seedlings were obtained, 18 of them carrying the molecular markers tagging the QTL and the transgene *BpMADS4*. Eight of these F1 seedlings flowered for the first time within 7 months after sowing and were used in reciprocal crosses with the cultivars ‘Topaz’, ‘Diwa’, and ‘Maloni Sally®’ in early 2010. Ninety one BC’1 seedlings were obtained, 24 of them carrying the molecular markers tagging the QTL and the transgene *BpMADS4*. Nine of these BC’1 seedlings have flowered so far (May 2011), i.e. within 7 months after sowing (December 2010). They have been pollinated with the apple cultivar ‘Royal Gala’ to continue the fast introgression of the fire blight resistance QTL from ‘Evereste’. Simultaneously, a Whole Genome Selection (WGS) based on microsatellite markers well-spanned over the apple genome is being applied on the 24 BC’1 seedlings carrying the QTL and the transgene *BpMADS4*.

## TRANS-GENERIC *S* ALLELE IN APPLE

**K.L. Bokszczanin<sup>1</sup>, A.A. Przybyła<sup>2</sup>, and A. Palucha<sup>3</sup>**

<sup>1</sup>Department of Plant Genetics, Breeding and Biotechnology, Warsaw University of Life Sciences – SGGW, 159 Nowoursynowska Str., 02-776, Warsaw, Poland

<sup>2</sup>Department of Pomology, Warsaw University of Life Sciences – SGGW, 159 Nowoursynowska Str., 02-776, Warsaw, Poland

<sup>3</sup>Genomed Sp. z o.o., Warsaw, Poland

For studies of self-incompatibility served Polish apple cvs: ‘Ligol’, ‘Odra’ and ‘Primula’. Using the RT-PCR method, cDNA was amplified on RNA isolated from styles. PCR products were cloned and sequenced. A trans-generic *S*-RNase allele, designated as *Skb* (GenBank accession no. EU443101), was discovered in cvs ‘Odra’ and ‘Primula’. Nucleotide sequence alignment revealed that *Skb*-RNase shows 98% identity to *SaucS19*-RNase from *Sorbus aucuparia* and 97% identity to *CmonS17*-RNase from *Crataegus monogyna*. The occurrence of extensive intergeneric hybridization among extant *Pyrinae* is considered since the deduced amino acid sequence of *Skb*-RNase from *M. x domestica* showed higher similarity to *CmonS17* from *C. monogyna*, *SaucS19*-RNase from *S. aucuparia*, *St* from *Malus transitoria*, *S5*-RNase and *S3*-RNase from *Pyrus pyrifolia*, and *S40*-RNase from *P. ussuriensis* than to *S*-alleles from *Malus x domestica* and all of them are grouped in the same cluster of phylogenetic tree. In ‘Ligol’, the agronomically most important cultivar in Poland, the *S2* and *S9* were identified.

## FRUITBREEDOMICS: A NEW EU-PROJECT TO IMPROVE FRUIT BREEDING EFFICIENCY

**F. Laurens<sup>1\*</sup>, M.J. Aranzana<sup>2</sup>, P.Arus<sup>2</sup>, J. Bonany<sup>3</sup>, L. Corelli<sup>4</sup>, A. Patocchi<sup>5</sup>, A. Peil<sup>6</sup>, B. Quilot<sup>7</sup>, A. Stella<sup>8</sup>, V. Troillard<sup>9</sup>, R. Velasco<sup>10</sup>, and E. van de Weg<sup>11</sup>**

<sup>1</sup>INRA Angers-Nantes Genetics and Horticulture (GenHort) JRU Beaucouzé, France

<sup>2</sup>IRTA Cabrils (Barcelona), Spain

<sup>3</sup>IRTA Mas Badia-La Tallada, Spain

<sup>4</sup>University di Bologna, Dipartimento Colture Arborre, Bologna, Italy

<sup>5</sup>ACW Research Station, Wädenswil, Switzerland

<sup>6</sup>JKI Institute for Breeding Research on Horticultural and Fruit Crops, Dresden, Germany

<sup>7</sup>INRA Avignon - Fruit and Vegetable Genetics and Breeding Unit, Avignon, France

<sup>8</sup>Parco Tecnologico Padano, Lodi, Italy

<sup>9</sup>INRA Transfert, Paris, France

<sup>10</sup>FEM-IASMA Research Centre, San Michele all'Adige (TN), Italy

<sup>11</sup>DLO, Plant Research International, Wageningen, The Netherlands

An international consortium gathering European and non European teams has designed FruitBreedomics, an ambitious research project, to improve the efficiency of fruit breeding by bridging the gap between scientific genetics research and application in breeding. The project will focus on apple and peach, two major fruits in Europe, but the tools and the knowledge gained will also benefit other species of the *Rosaceae* family *via* the strong ancestral relatedness among these species. The aim of FruitBreedomics is to provide the European fruit tree sector with cutting-edge breeding tools for the efficient and accelerated creation of new apple and peach varieties with excellent fruit quality characteristics, improved resistances to diseases and pests, and that can be grown in sustainable agriculture systems in the context of climate change. A major breeding tool to be developed is a validated pipeline for Marker Assisted Breeding and its implementation in ongoing commercial breeding programs. Towards this, the efforts will be directed to improve our understanding of the genetics of some major horticultural traits, develop innovative research tools to accelerate the breeding cycle, and efficiently find marker trait associations in breeding and GeneBank germplasm. Additionally, the project aims at increasing the accessibility of breeders to the genetic diversity present in GeneBank germplasm collections, thus contributing to widening the genetic basis of cultivated fruit trees. The collected data will provide precious genetic information on the pool of genitors and founders to be used in future breeding programmes. FruitBreedomics will use a multidisciplinary approach that includes genetics, genomics, ecophysiology and bioinformatics, and will liaison international partners with complementary expertises. From its start, the consortium aims at setting up a collaborative European network of breeders, GeneBank curators and industry representatives with the aim of rapidly and widely disseminating and implementing the obtained results among all interest European stakeholders.

## IMPLEMENTATION OF GENOMIC SELECTION IN APPLE BREEDING

**S. Kumar<sup>1\*</sup>, D. Chagné<sup>2</sup>, M.C.A.M. Bink<sup>3</sup>, R.K.Volz<sup>1</sup>, R.N. Crowhurst<sup>4</sup>, and C.J. Whitworth<sup>1</sup>**

<sup>1</sup>The New Zealand Institute for Plant & Food Research Ltd, Havelock North, Private Bag 1401, New Zealand

<sup>2</sup>The New Zealand Institute for Plant & Food Research Ltd, Palmerston North, Private Bag 11600, New Zealand

<sup>3</sup>Biometris, Wageningen University & Research Centre, PO Box 100, 6700AC, Wageningen, Netherlands

<sup>4</sup>The New Zealand Institute for Plant & Food Research Ltd, Mount Albert, Auckland, Private Bag 92169, New Zealand

The apple genome sequence and the availability of high throughput genotyping technologies provide an opportunity to speed up the development of new cultivars using a DNA-informed breeding strategy called genomic selection (GS). Genomic selection is an approach that utilizes thousands of genome-wide markers simultaneously to calculate genomic estimated breeding values (GEBVs) for complex traits. Thus, GEBVs offer plant breeders the opportunity to make selections of breeding parents and/or potential cultivars for further testing in commercial conditions. GS could substantially accelerate breeding cycles by making early selections, resulting in increased genetic gain per unit time. Following the publication of the reference genome of ‘Golden Delicious’, an international research consortium comprising The New Zealand Institute for Plant & Food Research Limited (PFR), the USA-led RosBREED and European-led FruitBreedomics research groups recently generated genome sequencing information for a further 27 apple cultivars. This has led to the development by PFR of the first chip comprising nearly 8,000 single nucleotide polymorphic (SNP) markers. This SNP chip is currently being used by PFR’s apple breeders to evaluate the GS technique as an alternative to phenotypic selection, primarily for complex traits. In this paper, we provide an overview of the implementation strategy for GS for apple fruit quality traits, preliminary results and a prediction of expected accuracy from such selection.



## THE SA-ARC PROCESSING PEACH BREEDING PROGRAMME: INTRODUCING MOLECULAR TECHNIQUES

**W-M. Pieterse**

ARC Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch, 7599, Republic of South Africa

e-mail: pietersew@arc.agric.za

In South Africa, peaches are used for canning, drying and – to a lesser extent – juicing and jamming as well as for the fresh market. The origins of the SA canning peach industry can be traced back to a peach from St. Helena, which was the progenitor of the Transvaal yellow non-melting cling type peaches. A chance seedling of this type was released as ‘Kakamas’ in 1932. This cultivar and ‘Early Dawn’, a mutation of the English cultivar ‘Duke of York’, were extensively used as parents in the early days of the breeding programme, indicating a narrow genetic base of the founder cultivars. These cultivars, together with ‘Muir’ and ‘Elberta’, imported from America, served as parents for breeding drying types. Desirable traits for the processing industries are uniform firm fruit, without a prominent suture or fruit point, small stones without stone extensions and no red coloration in the stone cavity or on the skin, no browning, high sugar content, skin with less or no pubescence, and a prolonged cropping season; and adaptation to medium to low chilling requirement is also important. Recent developments from the breeding programme include white - fleshed cling stone peaches for canning and yellow freestone nectarine cultivars suitable for drying. To address evolving industry needs, germplasm collections are being supplemented with material from foreign genebanks to expand the genetic base of the breeding programme and the intention is to use markers to identify and characterise appropriate breeding parents. Diverse segregating breeding progenies are being developed to underpin genetic studies on fruit quality traits, to identify QTLs and various loci involved and to develop markers. Concomitantly, breeders are being trained in molecular and mapping techniques to infuse the breeding programme with a molecular genetic approach to cultivar development.

## A STRATEGY FOR GENETIC MAPPING OF POLYPLOID KIWIFRUIT (*Actinidia*) SPECIES

P. Datson<sup>1\*</sup>, D. Mertton<sup>1,2</sup>, G. Tsang<sup>1</sup>, K. Manako<sup>1</sup>, L. Fraser<sup>1</sup>, and M. McNeilage<sup>1</sup>

<sup>1</sup>The New Zealand Institute for Plant & Food Research Limited, Private Bag 92169, Auckland 1142, New Zealand

<sup>2</sup>Friedrich-Schiller-Universität Jena, Jena, Germany

*Actinidia*, a diverse genus of dioecious vines, has a basic chromosome number of  $x = 29$  and the 55 species exhibit ploidy levels from diploid to octoploid. Two taxa, *A. chinensis* var. *chinensis* ( $2x$ ,  $4x$ ) and *A. chinensis* var. *deliciosa* ( $6x$ ) are grown for commercial fruit production. Most of the kiwifruit produced worldwide is from *A. chinensis* var. *deliciosa*. A genetic map of diploid *Actinidia chinensis* has been constructed using microsatellite markers, and mapping of tetraploid and hexaploid *Actinidia chinensis* varieties would assist in developing new cultivars. Interspecific hybrids can be formed between *Actinidia* species, including between species of differing ploidy. As microsatellite markers are generally transferable across *Actinidia* species, genetic mapping using progeny from interspecific crosses could reduce the number of same-sized alleles in progeny, and reduce the problem of dosage. To investigate whether tetraploid hybrids between *A. chinensis* var. *deliciosa* and *A. eriantha* would be useful for genetic mapping of *A. chinensis* var. *deliciosa*, parents and progeny were genotyped for microsatellite markers that mapped to LG2 in diploid *A. chinensis*. The dosages of alleles in the *A. chinensis* var. *deliciosa* parent were estimated from the proportion of progeny that exhibited the allele. Alleles that appeared to be present as a single copy in the *A. chinensis* var. *deliciosa* parent were mapped. Each different-sized *A. chinensis* var. *deliciosa* allele was scored as a separate marker, giving up to six markers per microsatellite. *A. eriantha* alleles were excluded. When markers were grouped in JoinMap 3.0, six groups for LG2 were created depending on the alleles that were present, before alleles were ordered, based on their recombination. This allowed mapping of each of the six homologs of LG2 in *Actinidia chinensis* var. *deliciosa*. Results suggest that there is some conservation of gene order of LG2 in *A. chinensis* var. *deliciosa* and *A. chinensis*. For this approach to be successful high marker density and a large mapping population would be required.

## TARGETED DEVELOPMENT AND MAPPING OF FUNCTIONAL MOLECULAR MARKERS IN AN APPLE ROOTSTOCK (*Malus pumila*) MAPPING PROGENY

**L. Antanaviciute<sup>1</sup>, F. Fernández-Fernández<sup>1</sup>, J.M. Dunwell<sup>2</sup>, N.H. Battey<sup>2</sup>, and D.J. Sargent<sup>1,3</sup>**

<sup>1</sup>East Malling Research, New Road, East Malling, Kent, ME19 6BJ, United Kingdom

<sup>2</sup>School of Biological Science, University of Reading, Whiteknights, Reading, Berkshire, RG6 6AS, United Kingdom

<sup>3</sup>Istituto Agrario San Michele all'Adige. Via E. Mach, 1 38010 S. Michele all'Adige (TN), Italy

e-mail: felicidad.fernandez@emr.ac.uk

The cultivated apple (*Malus pumila* Mill.) is an economically important crop, which is widely grown throughout the world. The identification of genes involved in traits of agronomic importance and the development of molecular markers for these genes is the key to the development of marker-assisted selection in breeding programs. Several genetic maps have been reported for apple, but the focus of these maps has been mainly on scion (fruit variety) crosses. In this investigation we aim to use information from the published apple genome sequence to develop intron-spanning primer pairs from the exons of *Malus* genes identified from within genetic regions of low marker density on a pre-existing SSR-based linkage map of an apple rootstock cross M.27 x M.116 (M432). Eighteen ‘gaps’ – regions larger than 10 cM containing no genetic markers - were identified for gene-specific primer design. BLAST analysis produced 2536 possible contig matches. The most significant 319 matches were selected and identified on 249 scaffolds. A total number of 165 gene-specific primers have been designed around the introns of genes located in these scaffolds. A set of 78 primers amplified single products in the parental genotypes whereas 33 amplified two products. Polymorphic markers developed within these scaffolds will increase marker density within regions. This communication details targeted development of markers for the improved saturation of the M432 apple rootstock linkage map.



**Oral session**

**GENETIC AND GENOMIC STUDIES OF RESISTANCE**



## PROGRESS IN PYRAMIDING DISEASE RESISTANCES IN APPLE BREEDING

**M. Kellerhals\*, L. Franck, I. Baumgartner, J.E. Frey, and A. Patocchi**

Agroscope Changins-Wädenswil, Schloss 1, P.O. Box, CH-8820 Wädenswil, Switzerland

Durability of disease resistance in apple under severe disease pressure has proved to be vulnerable. In many areas of middle and northern Europe it has become difficult to grow apple cultivars carrying the predominant *Vf* scab resistance only without a complete fungicide spraying program. Scab (*Venturia inaequalis*) isolates have emerged that were able to overcome this type of resistance. Similar resistance breakdown was reported for powdery mildew resistance such as PI-1 and PI-2 and others. We present an approach to combine in the same genotype several resistance genes towards apple scab (*Venturia inaequalis*) and to also incorporate resistance to powdery mildew (*Podosphaera leucotricha*) and to fire blight (*Erwinia amylovora*). Marker-Assisted Breeding (MAB) based on SSR and SCAR markers closely linked to resistance loci were used to detect progeny genotypes with combined resistance to scab, mildew resistance and enhanced resistance to fire blight. Fruit quality is the primary objective in our breeding program. To develop high quality varieties with durable disease resistance suitable for sustainable production systems, molecular marker analysis was applied, depending on the cross, at different stages in the breeding process and combined with a stringent phenotypic evaluation and selection. The first promising advanced selections were developed with pyramided resistance against scab and resistance against powdery mildew and fire blight. Those selections display commercial fruit quality. Problems associated to linkage drag derived from the original resistance donors in the pedigree will be discussed.

## OBSERVATIONS ON SCAB RESISTANCE IN INTERSPECIFIC PEAR SEEDLING FAMILIES

V. Bus<sup>1\*</sup>, L. Brewer<sup>2</sup>, and Ch. Morgan<sup>2</sup>

The New Zealand Institute for Plant & Food Research Limited

<sup>1</sup>Hawke's Bay Research Centre, Private Bag 1401, Havelock North 4157, New Zealand

<sup>2</sup>Motueka Research Centre, 55 Old Mill Road, RD3, Motueka 7198, New Zealand

Scab is a major disease of pear worldwide. The disease is caused by two species: *Venturia pirina*, which infects European pear, and *V. nashicola*, which infects Asian pear species. The host types are mutually exclusive to the *Venturia* species and this phenomenon is heavily exploited in the Plant & Food Research pear breeding programme for the breeding of scab-resistant pear cultivars. In 2008 and 2009, a total of 19 seedling families with a range of 0% to 100% European pear pedigree were screened in the glasshouse following artificial inoculation with *V. pirina*, the scab species present in New Zealand. The progenies showed a range of resistance reactions, mostly in the classes 0 (no symptoms) and 2 (necrotic/chlorotic reaction without sporulation). Some seedlings showed a hypersensitive response (class 1) or necrotic/chlorotic reactions with limited sporulation (class 3). As expected, the seedling progenies of predominantly European pear descent showed high proportions (78-91%) of susceptible seedlings, while 10 of the 19 families showed 100% resistant seedlings, with a further two families showing 98% and 94% resistance. We also show that the estimated ancestry of the parents is not necessarily a good predictor for the expected resistance segregations of the progeny, particularly where incompatibility effects are suspected of causing segregation distortions.



## DEVELOPMENT OF A CONTIG IN APRICOT CONTAINING THE PPV RESISTANCE LOCUS

**J.M. Soriano<sup>1</sup>, E. Zuriaga<sup>1\*</sup>, J. Martínez-Calvo<sup>1</sup>, C. Romero<sup>1</sup>, T. Zhebentyayeva<sup>2</sup>, A.G. Abbott<sup>2</sup>, and M.L. Badenes<sup>1</sup>**

<sup>1</sup>Instituto Valenciano de Investigaciones Agrarias, Apartado Oficial 46113 Moncada, Valencia, Spain

<sup>2</sup>Department of Genetics and Biochemistry, Clemson University. 100 Jordan Hall, Clemson, SC 29634, USA

e-mail: badenes\_mlu@gva.es

Sharka disease, caused by the *Plum Pox Virus* (PPV), is one of the major limiting factors for stone fruit production in Europe and America. Attempts to stop disease through eradication of infected trees were unsuccessful. Thus, introgression of resistance to PPV in the cultivars is the most important goal in the *Prunus* breeding programs. Beside of the difficulties inherent to the breeding perennial trees, phenotyping for sharka resistance is a time and labour consuming protocol, the major bottleneck in the breeding pipeline. In this context, identification of the genes involved in sharka resistance and/ or development of markers for assisted selection (MAS) will provide tools for early screening of seedlings and enhancing the breeding efficiency. In this study, using a linkage mapping approach and an apricot BAC library, we constructed a physical map of the resistance locus identified by Vera et al, (2011). The contig expand 2.16 Mb linked to PPV resistance. Alignments with the peach genome assembly v1.0 ([www.phytozome.org](http://www.phytozome.org)) showed potential candidate resistance genes for PPV.

## A CANDIDATE GENE OF *Malus x robusta* 5 FOR BREEDING TOWARDS FIRE BLIGHT RESISTANCE

**J. Fahrentrapp<sup>1\*</sup>, G. AL Brogini<sup>1</sup>, M. Kellerhals<sup>2</sup>, M. Malnoy<sup>3</sup>, A. Peil<sup>4</sup>, K. Richter<sup>5</sup>, and C. Gessler<sup>1</sup>**

<sup>1</sup>Plant Pathology Integrative Biology Zurich (IBZ), Swiss Federal Institute of Technology, ETH Zurich, Universitaetstrasse 2, LFW, 8092 Zurich, Switzerland

<sup>2</sup>Agroscope Changins-Wädenswil (ACW), P.O. Box, 8820 Wädenswil, Switzerland

<sup>3</sup>Fondazione Edmund Mach – FEM, Istituto Agrario San Michele- IASMA, Via Mach 1, 38010 S. Michele all'Adige (TN), Italy

<sup>4</sup>Julius Kühn-Institut, Federal Research Centre for Cultivated Plants, Institute for Breeding Research on Horticulture and Fruit Crops, Pillnitzer Platz 3a, 01326 Dresden, Gemany

<sup>5</sup>Julius Kühn-Institut, Federal Research Center for Cultivated Plants, Institute for Resistance Research and Stress Tolerance, Erwin-Baur-Strasse 27, 06484 Quedlinburg, Germany

In the last decades Fire Blight (FB) has become a damaging disease in all pome fruit growing areas worldwide except Australia. Most apple cultivars on the market are highly or moderate susceptible to the disease. In apple (*Malus x domestica*) cultivation conventional control measures like eradication and pruning but also the application of chemicals and bio-control agents do not prevent of severe outbreaks of the disease. Just the application of antibiotics (e.g. streptomycin, tetracyclines, kasugamycin) brings sufficient control. Particular selections of wild *Malus* species are highly resistant to FB. The use of these resistances in breeding new cultivars would be another possibility for disease control. However, *Malus x domestica* is highly heterozygous and cultivar self-incompatible with a generation cycle of min three-four years. Introgression of a single trait from a wild donor is a long and time-consuming process which includes several pseudo backcrosses to eliminate unwanted quality characteristics from the resistance donor. The resulting product will always be a new apple cultivar that has to be commercialized. Moreover Fire blight resistance tests need a particular set up and cannot be performed on seedling. The introgression of a particular trait can be speeded up by using molecular markers (molecular assisted selection, MAS), for which development the knowledge about the précis genomic location is indispensable. A step further is the identification and cloning of the resistance gene(s). These could be used to engineer transgenic or, even more consumer accepted, cisgenic apple plants of a popular commercial cultivar with the addition of the trait FB resistance. This would abbreviate the long time lag necessary for conventional breeding, introduction and commercialization a new cultivar with FB resistance. We will give insides on the region of resistance locus of the wild apple *Malus x robusta* 5 detected at linkage group 3 by QTL analysis and the putative candidate resistance gene.

## GENETICS OF RESISTANCE TO *Amphorophora idaei* IN RED RASPBERRY

**F. Fernández-Fernández<sup>1\*</sup>, L. Antanaviciute<sup>1</sup>, V.H. Knight<sup>1</sup>, J.M. Dunwell<sup>2</sup>, N.H. Battey<sup>2</sup>, and D.J. Sargent<sup>1,3</sup>**

<sup>1</sup>East Malling Research, New Road, East Malling, Kent, ME19 6BJ, United Kingdom

<sup>2</sup>School of Biological Science, University of Reading, Whiteknights, Reading, Berkshire, RG6 6AS, United Kingdom

<sup>3</sup>Istituto Agrario San Michele all'Adige. Via E. Mach, 1 38010 S. Michele all'Adige (TN), ITALY

e-mail: felicidad.fernandez@emr.ac.uk

Raspberry breeding aims to develop improved cultivars to satisfy market demands whilst allowing financially- and environmentally-sustainable production. The large raspberry aphid, *Amphorophora idaei*, transmits four viruses and thus its presence in raspberry plantings has a detrimental effect on fruit quality, yield and plantation longevity. Resistance to this vector has been central to the red raspberry breeding programme at East Malling for over 50 years and resistance genes ( $A_1$ ,  $A_{10}$ ,  $A_{K4a}$ ,  $A_{L518}$  and  $A_{cor}$ ) from different genetic backgrounds have been identified and introduced into our breeding lines. Selection pressure on the aphid, imposed by the widespread cultivation of resistant cultivars, has led to aphid populations overcoming  $A_1$ , and more recently,  $A_{10}$  resistance. As part of efforts to produce a more durable resistance, breeders have made crosses to combine several of these genes. However, most of them produce identical responses from the predominant aphid biotype so it is currently impossible to determine which resistance genes, and how many, are carried by breeding lines. Further, the genetic basis of the resistance itself have yet to be unravelled. A series of crosses designed to identify molecular markers linked to the various aphid resistance genes have been carried out and thus far,  $A_1$  and  $A_{10}$  have been allocated to linkage groups 3 and 7 respectively. This paper briefly summarizes current knowledge of *Amphorophora idaei*, introduces our research strategy on aphid resistance genetics in red raspberry, presents progress to date and outlines future work.

## FIRST RESULTS OF THE INITIATIVE “MONITORING OF *Venturia inaequalis* VIRULENCES”

A. Patocchi<sup>1\*</sup>, A. Auwerkerken<sup>2</sup>, S. Masny<sup>3</sup>, H. Nybom<sup>4</sup>, D. Pierre-Henri<sup>5</sup>, L. Tamm<sup>6</sup>, R. Vávra<sup>7</sup>, A. Peil<sup>8</sup>, B.A. Padder<sup>9</sup>, M. Lateur<sup>10</sup>, and V. Bus<sup>11</sup>

<sup>1</sup>AgroscopeChanginsWädenswil Research Station ACW, Schloss, 8820 Wädenswil, Switzerland

<sup>2</sup>Better3fruit, Steenberg 36, 3202 Rillaar, Belgium

<sup>3</sup>Research Institute of Horticulture, Pomology Division, Pomologiczna 18, 96-100 Skierniewice, Poland

<sup>4</sup>Balsgård-Department of Plant Breeding and Biotechnology, Swedish University Of Agricultural Sciences, Fjälkestadvägen 459, 29194 Kristianstad, Sweden

<sup>5</sup>AgroscopeChanginsWädenswil Research Station ACW, CP 1012, 1260 Nyon, Switzerland

<sup>6</sup>Research institute of organic agriculture (FiBL), Ackerstrasse, 5070 Frick, Switzerland

<sup>7</sup>Research and Breeding Institute of Pomology Ltd., Holovousy 1, 508 01 Horice, Czech Republic

<sup>8</sup>Julius Kühn-Institute, Federal Research Centre for Cultivated Plants, Institute for Breeding Research on Horticultural and Fruit Crops, PillnitzerPlatz 3a, D-01326 Dresden, Germany

<sup>9</sup>Division of Plant Pathology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, 191 121 Srinagar, Jammu and Kashmir, India

<sup>10</sup>Centre Wallon de Recherches Agronomiques, Breeding & Biodiversity Unit, Chemin de Liroux, 4- 5030 Gembloux, Belgium

<sup>11</sup>The New Zealand Institute for Plant & Food Research, Private Bag 1401, Havelock North 4157, New Zealand

e-mail: andrea.patocchi@acw.admin.ch

Apple scab, caused by *Venturia inaequalis*, is the major fungal disease in temperate regions throughout the world. Breeding of new apple cultivars with durable scab resistance will provide a sustainable solution to the problem. Durable, or at least more durable resistance can be achieved by pyramiding resistance genes into one genotype. Currently, however, breeders do not have sufficient information about what pyramids may confer durable resistance. While some commonly used resistance genes appear not to have been overcome to date, their limited temporal and race-specific exposure to the pathogen makes it difficult to predict their suitability for breeding. Global evaluation of the resistance genes for the corresponding virulences in the pathogen populations is required to obtain this information. Hence the international monitoring of virulences in different *V. inaequalis* populations on a continued basis was initiated. The collected data will be published yearly on the homepage of the initiative: [www.vinquest.ch](http://www.vinquest.ch). Over the last three years a network of 27 trap orchards comprising 16 differential hosts have been, or are in the process of being established in 14 countries. Currently, each orchard (105 plants in total) consists of 5 randomized blocks with one-tree plots of each host h(0) to h(15) (80 plants in total), and 5 placeholders (generally susceptible cultivars) per block that will be substituted in the future with genotypes carrying new scab resistance genes. We will present an overview of the initiative as well as the first apple scab incidence and severity data from the trap orchards of the partners that joined the network early.

## QTL MAPPING FOR RESISTANCE TO FIRE BLIGHT USING SEVERAL STRAINS RESULTING IN DIFFERENT HOST-PATHOGEN INTERACTIONS

Th. Wöhner<sup>1\*</sup>, I. Vogt<sup>1</sup>, K. Richter<sup>2</sup>, M.-V. Hanke<sup>1</sup>, C. Gessler<sup>3</sup>, G. Brogini<sup>3</sup>, J. Fahrenttrapp<sup>3</sup>, T. Garcia-Libreros<sup>4</sup>, A. Peil<sup>1</sup>, and H. Flachowsky<sup>1</sup>

<sup>1</sup>Julius Kühn-Institut (JKI), Bundesforschungsinstitut für Kulturpflanzen, Institut für Züchtungsforschung an gartenbaulichen Kulturen und Obst, 01326 Dresden, Germany

<sup>2</sup>Julius Kühn- Institut (JKI), Bundesforschungsinstitut für Kulturpflanzen, Institut für Resistenzforschung und Stresstoleranz, 06484 Quedlinburg, Germany

<sup>3</sup>Plant Pathology, Institute of Integrative Biology, ETH Zurich, Zurich, Switzerland

<sup>4</sup>AIT Austrian Institute of Technology GmbH, Seibersdorf, Austria

The bacterial fire blight caused by *Erwinia amylovora* (Burrill) Winslow et al. is one of the most destructive diseases in apple and pear production worldwide. To date no efficient strategies exist to cope with the disease in pomefruit production and the majority of cultivars dominating the marketplace are susceptible. Planting of resistant cultivars is the most promising strategy, but resistant cultivars with a fruit quality which can compete to leading cultivars on the marketplace are still missing. Breeding for fire blight resistance is therefore one of the major aims in nearly all pomefruit breeding programs. Different resources for resistance are known in apple wild species, but genetics of fire blight resistance is still poorly understood. First studies on mapping of fire blight resistance in the cross population 'Idared' × *Malus* × *robusta* 5 (Mr5) using the *Erwinia amylovora* strain Ea222 resulted in detection of a major QTL on linkage group 3 (LG3) of Mr5. In the present study we inoculated the cross population again with strains Ea11176 and Ea1189 (both avirulent to Mr5) as well as with strains Ea3049 (virulent to Mr5). Using the strains Ea11176 and Ea1189 we were able to confirm the QTL on LG3 of Mr5. In contrast, no QTL on LG3 of Mr5 was detectable after inoculation with strain Ea3049. In addition, a large number of different *E. amylovora* strains were evaluated on their virulence to Mr5 and other genetic resources of apple. Two additional strains were found, which were able to breakdown the fire blight resistance of Mr5. Surprisingly, all strains breaking the fire blight resistance of Mr5 were non-virulent to accessions of *M. fusca* and *M. baccata* suggesting a different mode of action in these genotypes.

## **cDNA-AFLP ANALYSIS OF DIFFERENTIALLY EXPRESSED TRANSCRIPTS IN PARTIAL RESISTANT APPLE CULTIVAR LEAVES INFECTED BY *Venturia inaequalis***

**H. Bastiaanse<sup>1\*</sup>, A. Pissard<sup>1</sup>, D. Mingeot<sup>2</sup>, Y. Muhovski<sup>2</sup>, J.-M. Jacquemin<sup>2</sup>, P. Lepoivre<sup>3</sup>, and M. Lateur<sup>1</sup>**

<sup>1</sup>Life Sciences Department, Breeding and Biodiversity Unit, Walloon Agricultural Research Center, Rue de Liroux, 4, 5030 Gembloux, Belgium

<sup>2</sup>Life Sciences Department, Breeding and Biodiversity Unit, Walloon Agricultural Research Center, Chaussée de Charleroi, 234, 5030 Gembloux, Belgium

<sup>3</sup>Plant Pathology Unit, Gembloux Agro-Bio Tech, University of Liège, Passage des déportés 2, 5030 Gembloux, Belgium

Scab, caused by the fungus *Venturia inaequalis*, is one of the most important diseases of apple (*Malus × domestica*). Whereas resistance genes (R-genes) governing qualitative resistance have been isolated and characterized, the biological roles of genes governing quantitative resistance, hypothesized to be more durable, are still unknown. This study aims to investigate molecular and biochemical mechanisms involved in the partial resistance of an old apple cultivar against *Venturia inaequalis*. Gene expression in both resistant and susceptible apple cultivars after scab inoculation was studied by using the cDNA-AFLP method (cDNA-Amplified Fragment Length Polymorphism). From approximately 10250 cDNA fragments detected on polyacrylamide gels, 252 bands were up- or down-regulated in the partial resistant cultivar ‘Président Roulin’ 48 hours after scab inoculation. From these bands, 135 appeared to be regulated only in the resistant cultivar compared to the susceptible one ‘Gala’. 224 fragments were constitutively expressed by the resistant cultivar ‘Président Roulin’ and were not affected by the fungal infection. All pathogen responsive fragments, and a part of the fragments showing constitutive expression were cloned, sequenced and compared to Genbank accessions using blastx. Homologies to known genes revealed several proteins belonging to the NBS-LRR and LRR-RLK classes of plant R genes. Other defense related genes were also identified and may play a role in the partial scab defense responses. Expression of these fragments will be verified by RT-PCR at six times after scab inoculation.

## TRANSCRIPTOMIC ANALYSIS OF TWO *Prunus* GENOTYPES DIFFERING IN WATERLOGGING RESPONSE REVEALS THE IMPORTANCE OF ANP AND HYPOXIA-ASSOCIATED OXIDATIVE RESPONSE

M.J. Rubio-Cabetas<sup>1\*</sup>, M.L. Amador<sup>1</sup>, C. Pons<sup>2</sup>, C.A. Martí<sup>2</sup>, and A. Granell<sup>2</sup>

<sup>1</sup>Unidad de Fruticultura CITA – Gobierno de Aragón, Avda. de Montañana 930, 50059 Zaragoza, Spain

<sup>2</sup>IBMCP CSIC-Universidad Politécnica, E-48022 Valencia, Spain  
e-mail: mjrubioc@aragon.es

Plant breeding in stone-fruit trees is mainly focused to increase rootstock tolerance to abiotic stress. The molecular response program to hypoxia caused by waterlogging involves many genes, and at least 20 anaerobic polypeptides (ANPs) have been described in different sugar metabolism pathways. Experiments carried out to unravel transcriptional regulation in response to hypoxia treatment of two *Prunus* rootstocks will be presented. *In vitro* plants were submitted to hypoxia and normoxia conditions. The hypoxic treatment was carried out in airtight chambers with 3% O<sub>2</sub>, 0.03% CO<sub>2</sub> and 97% N<sub>2</sub> gas composition for 2 h and 24 h. For each experimental point (0, 2h and 24 h) a sample representing three sets of roots were taken for each genotype. A ChillPeach microarray representing 4261 peach unigenes was hybridized with cDNA from root tissues of Myrobalan ‘P.2175’ (*P. cerasifera*) and ‘Felinem’ hybrid (*P. amygdalus* x *P. persica*), these corresponding to waterlogging-tolerant and sensitive plants respectively. A SAM multiclass analysis of the gene expression dataset was used to analyse globally the effect of hypoxia over treated roots compared to control roots at different times. A total of 2442 genes were found differentially expressed. Out of them, 916 genes show already differences in expression levels between genotypes at time 0 with 434 of those being more highly expressed in the tolerant genotype. A hierarchical clustering analysis revealed a number of genes encoding ANPs, as well as genes previously reported as induced by H<sub>2</sub>O<sub>2</sub>, and suggest a possible crosstalk between the signaling pathways for hypoxia and oxidative stress. Among the ANPs the one encoding an Alanine Aminotransferase seems to play even a more important role than other important players such as Alcohol Dehydrogenase and Pyruvate Decarboxilase in terms of energy production as it would prevent the accumulation of Pyruvate. Among the antioxidants enzymes differences has been found in the activities in different cell compartments, chloroplast, mitochondrial, etc.





# Poster sessions



**Poster session**

**FRUIT BREEDING PROGRAMMES**



## SELECTION FOR FUTURE ON PEACH CULTURE (*Prunus persica* L. [Batsch]) IN ROMANIA

A. Baci<sup>1\*</sup>, I. Botu<sup>1</sup>, S. Cosmulescu<sup>1</sup>, M. Gruia<sup>1</sup>, and L. Dumitru<sup>2</sup>

<sup>1</sup>University of Craiova, Faculty of Horticulture, A.I. Cuza, 13,  
200585 Craiova, Romania

<sup>2</sup>Research Station for Fruit Growing Constanta  
e-mail: aurelian.adrian@gmail.com

Common peach (*Prunus persica* L. [Batsch]) present diversity of forms and varieties, being involved in obtaining of peach cultivars. Inter- and intraspecific hybridization, natural and directed, had created over time a large number of cultivars, which are distinguished by morphological features and characteristics of fruit trees. Reducing size of the trees is a priority objective in tree improvement, designed to intensifying culture, along with a new architectural achievement of the trees, to simplify the maintenance of culture and technology to create better conditions for full exploitation of biological potential and fruit coloring. By controlled hybridization of dwarf genotypes with standard cultivars can be obtained trees under medium size, with fruits intensively colored, pulp consistent and pleasant taste. In the research program conducted at the University of Craiova and research stations for fruit growing in the south of Romania, have been obtained and proposed approval for two cultivars of dwarf nectarine ('Minival' and 'Nectval') and peach ('Anemona'), to complete peach assortment in Romania. The genotypes were studied in phenological terms, have been carried out physicochemical assessments on productivity, ripening time and the reaction to diseases and pests.

## UTILITY OF WILD GERMPLASM IN OLIVE BREEDING

**T. Klepo, R. De la Rosa, L. León, and A. Belaj\***

IFAPA Centro Alameda del Obispo, Avda. Menéndez Pidal, s/n, 14004, Córdoba  
e-mail: [angelina.belaj@juntadeandalucia.es](mailto:angelina.belaj@juntadeandalucia.es)

Most of the breeding works carried out in olive up to date has been based on the current ancient cultivars that have been produced by empiric selection of the growers through centuries. However, the high level of variability found in wild olive populations indicates that they could have genes of interest for obtaining olive cultivars adapted to the new trends of olive growing. In order to test the suitability of wild olives for breeding, we compared the segregation of an open pollination progeny of a wild olive located in a forest in Cadiz, Southern Spain, with a progeny obtained from free pollinization of a 'Picual' tree located in the World Olive Germplasm Bank of Cordoba, Spain. As expected, the wild progeny beared fruits of low weight and oil content. However, surprisingly, wild seedlings showed a significant lower juvenile period than the ones coming from 'Picual' open pollination progeny. This trait is very important for breeders and, quite likely, has not been a criteria of selection of the current cultivars. Additionally, seedlings from two progenies coming from the cross of 'Picual' with two wild olive trees have been evaluated. Fruit characteristics were intermediate between 'Picual' seedlings and wild seedlings. However, a great heterosis effect has been observed as those seedlings had much higher height and trunk size than seedlings coming from the cross of two cultivars. Accordingly, the average juvenile period of 'Picual' x wild progenies were much lower than the one observed in the progenies of the cross of two cultivars. All these results suggest the utility of wild germplasm in olive breeding that needs to be further investigated.

## BREEDING OF RESISTANT STRAWBERRY CULTIVARS FOR ORGANIC FRUIT PRODUCTION: PRELIMINARY RESULTS WITH *Botrytis cinerea*

M. Bestfleisch<sup>1\*</sup>, M. Höfer<sup>1</sup>, K. Richter<sup>2</sup>, M.-V. Hanke<sup>1</sup>, E. Schulte<sup>3</sup>, and H. Flachowsky<sup>1</sup>

<sup>1</sup>Julius Kühn-Institut (JKI), Bundesforschungsinstitut für Kulturpflanzen, Institut für Züchtungsforschung an gartenbaulichen Kulturen und Obst, 01326 Dresden, Germany

<sup>2</sup>Julius Kühn- Institut (JKI), Bundesforschungsinstitut für Kulturpflanzen, Institut für Resistenzforschung und Stresstoleranz, 06484 Quedlinburg, Germany

<sup>3</sup>Bundessortenamt - Prüfstelle Wurzen, 04808 Wurzen, Germany

Organic fruit production is mostly hampered by fungal and bacterial plant diseases. Especially the grey mould disease caused by the fungus *Botrytis cinerea* and the bacterial angular leaf spot disease caused by *Xanthomonas fragariae* are two of the most important diseases in organic strawberry production. Pesticides are not permitted in organic farming and indirect plant protecting measures such as cultivation methods and application of plant strengthening products are only less effective. Planting of resistant cultivars seems to be the most promising strategy to improve the productivity in organic strawberry production. Although commercially cultivated strawberry cultivars differ in their susceptibility to these diseases no resistant cultivars are available on the marketplace. On this account we started a program on evaluation of strawberry genetic resources for their resistance/susceptibility to *B. cinerea* and *X. fragariae*, respectively. We collected different strains of each pathogen and tested their virulence to a defined set of strawberry genotypes. Subsequently, we established methods for artificial fruit/leaf inoculation in the greenhouse for both pathogens and started the evaluation of strawberry cultivars and wild species of the Fruit Gene Bank Dresden-Pillnitz. Based on the results resistant/tolerant genotypes will be selected and used in test crosses to determine the general and specific combining ability. The results of the test crosses will lead to the identification of genotypes that are suitable for a targeted resistance breeding against *B. cinerea* and *X. fragariae*, respectively.

## BREEDING OF HIGHBUSH BLUEBERRY IN LITHUANIA

**L. Česonienė\* and R. Daubaras**

Kaunas Botanical Garden of Vytautas Magnus University, Ž.E. Žiliberio 6, Kaunas LT-46324, Lithuania  
e-mail: laimac@hotmail.com

Growing of half highbush blueberry in Lithuania has been limited till the present time. One of the main reasons for such situation was absence of local cultivars. The collection of half highbush blueberry germplasm and respective breeding programme were started at Kaunas Botanical Garden of Vytautas Magnus University in 1993. Fifty seedlings were examined in the years 1993 - 2010. The seedlings № 11, № 16, and № 17 were selected for further cultivar testing. The results were compared with the same characteristics of the cultivar Putte. Fruiting potential of Lithuanian half highbush blueberry accessions was determined, taking into account the following characteristics: number of berries and number of simple clusters in a composite cluster, average berry mass and volume. Productivity of investigated seedlings was determined too. The seedling № 17 was distinguished for the biggest clusters with the largest number of berries and the largest number of simple clusters in a composite cluster. The average berry mass of selected seedlings was smaller than berry mass of the cultivar 'Putte'. Ripening period of all investigated seedlings was long. Berries of the seedlings № 11 and № 16 were harvested completely during 2 - 3 pickings, whereas harvesting berries of the seedling № 17 was completed in 3 - 4 pickings. The № 17 appeared the most productive, its yield reached from 1.8 to 3.3 kg in different years. Yields of the seedlings № 11 № 16 were significantly lower.



## **PEACH BREEDING PROGRAMME FOR NEW AND DIFFERENT TRAITS. POMOLOGICAL AND PHENOLOGICAL DATA ANALYSIS WITH A RANKING METHOD**

**G. Cipriani\*, L. Conte, D. Bevilacqua, A. Di Cintio, M. Terlizzi, and A. Sartori**

CRA-FRU Centro di Ricerca per la Frutticoltura, via di Fioranello 52 – 00134 Roma  
e-mail: guido.cipriani@entecra.it; alisea.sartori@entecra.it

The main purposes of the breeding programme developed at CRA-FRU are presented. These include the selections of new types of peach and nectarines. We considered traits specifically limited to the fruits like: flat form, low-acid taste, deanthocyanic and bloody flesh. All the descriptive (pomological, phenological, agronomical) data of about 20 years of the 535 single advanced selections (365 peaches and 170 nectarines) were collected and elaborated with a ranking method, in order to point out the best performing genotypes. The most important characteristics in the evaluation were: weight, shape and over-colour (with a higher ranking score) then: taste, yield and hardness, fruit symmetry and ripening period. Among the 365 peach selections, 42 flat peaches, 21 deanthocyanic, 38 bloody fleshed and 4 low acid taste were identified. Among the 170 the nectarines, 8 flat, 1 deanthocyanic, 2 low acid, 21 bloody fleshed selections were detected. In order to select the most promising accessions a fruit type specific multi-criteria ranking method was adopted: for the flat form, the absence of cracking and skin and flesh damages; for low acid-taste the Soluble Solid Content (SSC); for the deanthocyanic flesh the white-cream skin and flesh coloration and the perception of low acid taste; for the bloody flesh, the red colour for more than 35% presence in the flesh, the SSC content and the lack of cracking and rust were respectively considered. The last step was to exclude the contemporaneous selections and those that ripen at the same time of the licensed and commercial cultivars. It was possible to discard about the 90% of the total selections. The pomological and phenological characteristics of the top peach selections are presented.

## SELECTION OF EUROPEAN CRANBERRY IN LITHUANIA

**R. Daubaras\* and L. Česonienė**

Kaunas Botanical Garden of Vytautas Magnus University, Ž.E. Žilibero 6, Kaunas, LT-46324, Lithuania

In order to optimize collection strategy and improve economically important peculiarities of *Vaccinium oxycoccos* comprehensive morphological and genetic investigations are necessary. For this reason collection and preservation of European cranberry genetic resources is necessary. The aim of this study was to investigate morphological peculiarities, productivity, and seasonal development of European cranberry clones collected in Lithuanian strict protected reserves. Collecting of cranberry clones during expeditions *in situ* was based upon distinguishing of samples conspicuous in high yield, berry shape, colouration and size. It was found that detected *in situ* properties of *V. oxycoccos* were retained under the *ex situ* conditions. Investigations revealed statistically reliable differences among cranberry clones in respect of berry and leaf size, productivity, and seasonal development. Integrated evaluation of morphological and economical peculiarities determined the selection of the most productive clones which were characteristic of large berries as well. The results of morphological and genetic diversity determine selection of clones with valuable properties and preservation of European cranberry germplasm *ex situ* at Kaunas Botanical Garden collection. The conspicuous by good productivity and large berries clones 99-Ž-11, 96-Ž-10, and 99-Ž-10 were selected and proposed for cultivar approval.

## ACHIEVEMENTS IN APPLE BREEDING AT ZHENGZHOU FRUIT RESEARCH INSTITUTE, CAAS

**G. Guo\*, Y. Zhenli, Z. Hetao, and Z. Ruiping**

Zhengzhou Fruit Research Institute, Chinese Academy of Agricultural Sciences,  
Zhengzhou, Henan, 450009 China  
e-mail: guonan0318@163.com

In 1960 the research programme on apple breeding and cultivar testing was started at Zhengzhou Fruit Research Institute of the Chinese Academy of Agricultural Sciences, located in Central China. Since then more than 420 varieties and strains have been collected and evaluated, with aim to select the best adapted to cultivation under local environmental conditions. From about 160 crossbreeding combinations more than 80,000 seedlings were obtained and screened. The best 50 selections have been tested in the commercial orchards. So far 12 new cultivars were released and tested in major apple production areas of China. 'Huaguan', obtained from the combination of Golden Delicious and Fuji, has been widely grown in China on the total area of about 50 thousands hectares and was tested in 10 countries through the international agency. In the middle of 1990s, a project for breeding early ripening cultivars was initiated. Some new early cultivars and selections have been obtained. 'Huashuo' ('NY543' × 'Huaguan') was released in 2009, with good color and large fruit size. The fruit flavor is sour-sweet with weak aroma. It has good texture, crisp and juicy. The fruit quality can be retained in cold store for at least 3 months; it has also a long shelf life. Fruit maturity time is 10 days early than 'Gala'. Some other new cultivars were released such as 'Huamei' ('Gala' × 'Huashuai'), 'Huayu' ('Mato' × 'Royal Gala'), for early fruit season, with good characteristics in comparison with 'Gala'. All of them are tested under different conditions of main apple growing areas of China.

## ALMOND BREEDING IN IRAN AND ITS ACHIEVEMENTS

### A. Imani

Horticultural Research Department, Seed and Plant Improvement Institute (SPII), Karaj, Iran, P.O.Box4119  
e-mail: imani\_a45@yahoo.com

Almond breeding program in Iran with aims of achievement to cultivars with desirable pomological and vegetative characteristics, and suitable performance particular resistant to frost damage can be divided to 2 stages: At first stage, collection and evaluation of native germless and adaptation of foreign cultivars was performed in different region of in Iran. In this period (1953 - 1966) in addition to identification of selected superior genotypes, they were evaluated with commercial cultivars in Research Stations of Azarshahr (Tabriz), Kamal-Shahr (Karaj), Bstam (Shahrood), Zargan (Shiraz) and Toroq (Mashad). The result of this research was selection of 1 selection of local (Sahand almond) and 3 commercial cultivars ('Ferragnes', 'Ne Plus Ultra' and 'Nonpareil'). These cultivars were mostly late blooming, high yield and quality. At the time of evaluating foreign cultivars and local selected genotypes, a crossing program in 1967 in Research Station of Sahand (RSS) to obtain late flowering cultivars was started. In finally, 3 commercial hybrids ('Azar', 'Shekofeh' and 'Harir') were released from this program. Also hybridizations in 1995 using released hybrids as parents in RSA carried out and recently 2 commercial hybrids ('Araz' and 'Eskandar') were released from this program in 2010. At second stage to obtain new cold resistant, late flowering cultivars and adapted to a rang of adverse climatic with desirable vegetative characteristics, and suitable nut characteristics, a program of hybridization between almond cultivars (emphasis on local selections) carried out in 1998 in Research Station of Kamalshahr (Karaj). In this research 1120 hybrids obtained from 20 different combinations of crosses. The primary results revealed 20 promising hybrids based on grading and assigning value, with respected to the importance and effectiveness of traits in production, especially flowering time. Late frost spring resistance, potential fruit set; bearing habitat and trees safety was selected for advance evaluation. In finally, two new late-blooming almond cultivars, 'Aidin' and 'Saba' were released in 2011. Based on these results, almond research program on variety and rootstock breeding has been provided for future in which this article will be described.

## TRANS-BORDER PEAR BREEDING PROGRAM FOR SCAB TOLERANCE AND NOVEL TRAITS

**M. Lateur<sup>1\*</sup>, J-B. Rey<sup>2</sup>, A. Rondia<sup>1</sup>, M. Lascoste<sup>2</sup>, and R. Stiévenard<sup>2</sup>**

<sup>1</sup>Department of Life Sciences – Breeding & Biodiversity Unit, Walloon Agricultural Research Centre, Rue de Liroux 4, 5030 Gembloux, Belgium

<sup>2</sup>Regional Centre of Genetic Resources, Ferme du Héron, Chemin de la Ferme Lenglet, 59650 Villeneuve d'Ascq, France

e-mail: lateur@cra.wallonie.be

Although more than 1800 pear accessions have been collected in old collections, gardens and regional pastured standard tree orchards by both the French Regional Genetic Resources Centre (ENR-CRRG) and the Walloon Agricultural Research Centre (CRA-W), only four commercial cultivars represents more than 65% of the European pear production. There is a dramatic erosion of the genetic diversity of the commercial pear sector which is typically illustrated by the Belgian case, as the fourth most important pear producer in Europe, 87% of its production relies on the *quasi* monoculture of the cv 'Conference'. Very curious is the fact that in opposite with apple, the most important cvs are still very old historical cultivars and the very few new cvs have all difficulties to merge on the market. Face to this context, a common breeding program was decided to start with as major objectives to create new cultivars more tolerant to scab – especially the twig infection form. As more than 70% of the spray treatments are devoted to scab, such objective becomes of utmost importance for the next future. The second objective is to develop bright coloured fruits that contrast with the gloomy face of 'Conference'. The breeding program aims also to use more intensively our large diversity of characters expressed by local old cvs and to use the best performed ones as parents. Some results of crosses involved for scab resistance after both controlled inoculations and subsequently in an unsprayed nursery are presented and discussed and especially the correlation between results from early tests in greenhouse *vs* nursery and from scab on leaves *vs* scab on wood.

## RED PEAR BREEDING IN CHINA

X. Li

Zhengzhou Fruit Research Institute, Chinese Academy of Agricultural Sciences

In China, 13 *Pyrus* species have been described and more than 3,000 cultivars have been recorded. Red-skinned cultivars are about 50 and belong to *P. pyrifolia* Nakai, *P. ussuriensis* Maxim. and *P. sinkiangensis* Yu. In the recent 20 years, using as a parent special Chinese pear with crispy flesh and red-skinned, many new red-skinned cultivars have been released, such as 'Hongxiangsu', 'Mantianhong', 'Meirensu', and 'Hongsucui', bred by Zhengzhou Fruit Research Institute, Chinese Academy of Agricultural Sciences during the 1990s. These new cultivars have very attractive fruits, good eating quality and in some areas became the main cultivar, e.g. Red-skinned cultivar 'Hongxiangsu' derived from a cross 'Korla's Xiangli' × 'Zhengzhou Eli'. It matures in early September at Zhengzhou, Henan province and has the outstanding characters. The fruits are medium size, on average 220g, elongate. The skin is greenish-yellow with purplish-red blush. The flesh is white, crispy, very juicy, sweet and aromatic, with SSC of 12%-13%. Fruits can be stored for 1-2 months in the common room temperature. In the recent years a number of more excellent monoclonal have been selected. In the meanwhile, molecular assisted breeding was done. A genetic linkage map was constructed from F<sub>1</sub> population comprising 94 seedlings derived from a cross between 'Chonghuadali' × 'Shinseiki' using mapmaker/exp3.0. The map consisted of 19 SSR markers and 315 SRAP markers which belong to 18 linkage groups and spans 1300cM with an average genetic distance of 3.9cM. The interval mapping was used to identify quantitative trait loci (QTL) associated with fruit developing period and fruit shape index in the F<sub>1</sub> population. The results indicated 7 QTLs linked to the two fruit traits, of which 2 QTLs were major effect genes (LOD $\geq$ 3.5). The two QTL regions were found on LG2 and LG17.

## **DEVELOPING ECONOMICALLY AND CULTURALLY VIABLE APPLE CULTIVARS FOR THE MIDWEST UNITED STATES: RESULTS FROM THE MIDWEST APPLE IMPROVEMENT ASSOCIATION BREEDING PROGRAM**

**D.D. Miller**

Department of Horticulture and Crop Science, Ohio State University/OARDC, 1680 Madison Avenue, Wooster, OH 44691 USA

The Midwest Apple Improvement Association (MAIA) is a grower-driven apple breeding program with the help of land grant institutions in the Midwest US. The majority of the 100 grower members are from Ohio, Indiana, Illinois and Kentucky. The main breeding objectives of MAIA are: high eating quality and environmental adaptability – including reliable and productive cropping potential and resistance to biotic (fireblight, scab) and abiotic (late spring freezes) stressors. The program began in 1998 and involves growers making crosses and growing seedlings at their orchards. 2nd test selections have been presented to consumers annually at a food show to test the consumer acceptance and potential in the apple market. These untrained consumers carried out the sensory evaluations of the new selections in comparison with commercial cultivars. Consumers filled out questionnaires with questions about their visual and taste preferences for apple cultivars/selections along with background questions that helped understand their demands and apple purchasing behavior. Consumer sensory perception was not closely correlated with instrumentally measured qualities (fruit size and shape, skin color, flesh firmness, soluble solids, acidity) of the fruit. Texture and flavor were the two most influential cultivar features in overall consumer preference. Sensory evaluations revealed high preference and potential marketing value of several new MAIA selections; some of them were uniformly liked by a wide range (age, gender, etc.) of consumers, while other selections had unique flavor characteristics that resulted in bimodal consumer ratings. While selections with a wide consumer acceptance have great potential for supermarkets and grocery stores, selections with divided consumer preference (liked by only a specific group of consumers) have better potential for direct markets.

## ***Hansabred* – A NEW EUROPEAN STRAWBERRY BREEDING AND RESEARCH COMPANY**

**K. Olbricht\*, U. Gerischer, A. Ludwig, S. Kraege, S. Walpole, P. Walpole, B. Casas, A. Darbonne, A. Vissers, and H. Obers**

Hansabred GmbH & Co. KG, Radeburger Landstraße 12, 01108 Dresden, Germany  
e-mail: k.olbricht@hansabred.org

In 2008, *Hansabred GmbH & Co. KG* was founded as a European strawberry breeding and research company the shareholders of which are four widely known European companies: *Kraege Beerenpflanzen* (Germany), *Vissers* (Netherlands), *R. W. Walpole Strawberry Plants Ltd.* (UK) and *Plantas de Navarra, S.A.* (Spain). 250 years of strawberry breeding have led to numerous so-called tunnel effects which resulted in susceptibilities to pathogens and in aroma patterns with low or even zero key volatile concentrations. In this contribution, important aspects of the *Hansabred* breeding programme are portrayed: high resistance to different pathogens and improved strawberry flavour. A large germplasm collection which originated from the "Professor Staudt Collection" is available with more than 260 accessions of wild species and more than 100 cultivars. This background allows innovative pathways in strawberry breeding. Today, several vigorous clones with excellent flavour are in trial already. *Hansabred* has initiated a project network together with fruit processing companies as well as national and international research institutes and universities.



## ***Vf* - SCAB RESISTANT APPLE CULTIVARS FROM 35-YEARS APPLE BREEDING PROGRAMME IN WARSAW**

**E. Pitera**

Department of Pomology, Warsaw University of Life Sciences-SGGW, 159 Nowoursynowska Str., 02-776 Warsaw, Poland

Scab resistant apple trees breeding programme at Warsaw Agricultural University was started in 1975. Its main goals were: resistance to scab, winter hardiness, high fruit quality and productivity. Since 1988 tolerance against powdery mildew was also included. The most important source of low susceptibility to mildew in Warsaw is our *Vf* scab resistant clone U 211 ('Primula' open pollinated). During last 20 years the purpose of the most crosses was creation of commercial cultivars with long storage of fruits. Since 1995 three scab resistant cultivars were released: 'Witos', 'Sawa', and 'Waleria'. Additional scab resistant selections U 7979 (proposed name 'Chopin') is in the second phase of assessment. From the three mentioned cultivars the most successful for Polish climate conditions is 'Sawa' ('Fantazja' × 'Primula'), but its main disadvantage is short storability of fruits. U 7979 ('Chopin') originated from cross of 'Granny Smith' and U 211 made in 1997. The preliminary observations showed, that trees of U 7979 are moderately vigorous, very precocious and productive. Fruits are green with occasionally pale pink blush, medium in size. Apple picking time is middle October and fruits can be stored until spring in a cool storage. U 7979 is a dessert apple, but can be also used for salads and processing. It can be recommended for sustainable fruit production. No symptoms of scab on 'Witos', 'Sawa', 'Waleria', and U 7979 ('Chopin') were noted till now.

## WIDENING HAZELNUT (*Corylus avellana* L.) GENETIC RESOURCES AND SELECTION CRITERIA FOR THE GENETIC IMPROVEMENT OF PHENOLOGICAL, PEST RESISTANCE, AND SEED SIZE TRAITS

G. Catarcione<sup>1</sup>, D. Vittori<sup>1</sup>, S. Bizzarri<sup>2</sup>, E. Rugini<sup>1\*</sup>, and C. De Pace<sup>1</sup>

<sup>1</sup>Dept. of Science and Technologies for Agriculture, Forestry, Nature, and Energy, University of Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy

<sup>2</sup>ARSIAL – Agenzia Regionale per lo Sviluppo e l’Innovazione dell’Agricoltura del Lazio, Strada Cassia Cimina Km 16400 - 01037 Caprarola (VT)

e-mail: rugini@unitus.it

Although common garden collections of hazelnut (*Corylus avellana* L.) landraces are available, and amount of variation for several plant and fruit traits are known, it is not clear if that diversity is the largest phenotypic diversity achievable in the cultivated gene-pool or if there is opportunity to further enlarge and improve the trait diversity for breeding new hazelnut clonal varieties. Landraces are mainly the results of clonal propagation by suckers from plants producing mutated shoots. However little information are available on the effectiveness of clonal propagation of plants raised from seedlings of either half-sib or full-sib progenies to develop new genetic diversity useful for trait enhancement. Therefore, we compared phenotypic diversity for phenology, pest resistance, and seed size traits in a representative collection of hazelnut landraces and in half-sib and full-sib progenies from some of the landraces. The aim was to assess the amount of variation stemming from open pollination or biparental crossing of hazelnut accessions in comparison to the diversity available among accessions representative of the hazelnut germplasm grown in different countries. All the materials were developed through common garden experiments to facilitate comparisons. Analysis was carried out for the traits showing good heritability as estimated from the parent-offspring regression analysis involving eight parental accessions and their half-sib progenies. Significant regression coefficient ( $b = \frac{1}{2} h^2$ ) was estimated for time of beginning of anthesis, time of bud sprouting, and nut length; the proportion of buds infested by big-bud mite showed good inheritance when evaluated on the progenies from parents expressing extreme high (Tonda di Giffoni; Tonda Gentile delle Langhe; Sivri) and extreme low (Longue d’Espagne; Nocchione) mite infestation; duration of anthesis, nut width, and seed weight did not show significant  $b$  values. Diversity between accessions were similarly clustered using molecular markers (AFLP and SSR) and the phenotypic values for the traits with high heritability. The higher divergence was found for the group of accessions from Turkey (3), Italy (15), USA (2) and UK (3). The accessions from Spain (6) overlapped those of the former groups. The diversity among accessions within clusters was generally small, and those from Italy were included in three main divergent clusters with entries from Sicily (which included “Nocchione”) in one cluster and entries from Campania and Latium (i.e. Tonda Gentile Romana), in the others. Discriminant analysis of the accessions grouped according to their geographic origin, clearly confirmed this property as a major factor of the phenotypic and molecular diversity. When parents were chosen from clusters of accessions divergent for molecular and phenotypic traits, such as “Tonda Gentile Romana” and “Nocchione”, their full-sib progeny, compared to the whole range of accessions in the germplasm collection, gave similar or even significant larger range of diversity for the examined high heritable

traits. These results suggest that divergence analysis among hazelnut accessions for (neutral) molecular markers as well as for traits that show good heritability, is useful for choosing parental accessions to produce genetically variable progenies to select for new combinations of phenological, pest resistance and seed size trait combinations.



**Poster session**  
**NEW CULTIVARS**



## DETERMINATION OF CLUSTER CHARACTERISTICS AND POMOLOGICAL PROPERTIES OF SELECTED CLONES OF ELHAMRA (*Vitis vinifera* L.) GRAPE VARIETY

A. Dardeniz<sup>1\*</sup>, M. Seker<sup>1</sup>, K. Kahraman<sup>2</sup>, G. Arda<sup>1</sup>, and L. Türker<sup>1</sup>

<sup>1</sup>University of Çanakkale Onsekiz Mart, Faculty of Agriculture, Department of Horticulture, Canakkale, Turkey

<sup>2</sup>Ataturk Central Horticultural Research Institute, Yalova, Turkey

Clonal selection studies on grapes have been carried out consisting of 37 cultivars and selection studies were completed in 24 ones in Turkey. Elhamra (Hönüsü) variety is a popular cultivar which grown in all grape production regions. It is grown extensively in East Mediterranean and South East Anatolia regions of Turkey. The variety is late maturing with short pruning characteristics. The flowers are functionally female, so the variety requires pollinator. Different Elhamra clones were determined during selection studies which started in 2008 in Canakkale province of Turkey and some of them marked for promising characteristics. The selected 3 clones were evaluated for their bunching and pomological characteristics in 2009 and 2010 harvesting seasons. According to the obtained results, the genotypes showed different characteristics for ripening time and some fruit parameters as follows: Clone 1 has large deep red to violet-black berries, medium to moderate cluster, tends to parthenocarpy and late maturing characteristics (mid October). Clone 2 has large deep red to violet-black berries, large cluster, tends to parthenocarpy and late maturing characteristics (mid October). Clone 3 has small to medium deep red to violet-black berries, medium cluster, less tends to parthenocarpy and late maturing characteristics (end of October). The results showed Elhamra variety has genotypic variation and detailed selection studies should be carried out.

## **BETTER3FRUIT: COMERCIAL RELEASES AFTER 10 YEARS**

**I. De Wit\*, A. Auwerkerken, and A. Van den Putte**

Better3fruit, Steenberg 36, 3202 Rillaar, Belgium

Better3fruit, a private company based in Belgium, runs one of the biggest apple and pear breeding programs in the world. Each year we create 10.000 seedlings of apple and of pear. At the time of its foundation, in 2000, Better3fruit took over the apple breeding program of the Catholic University of Leuven. In 2006 Better3fruit started a pear breeding program in the Czech Republic. During its short existence Better3fruit has already released a significant number of apple varieties successfully. The first apple varieties were released in 2002: Nicoter/Kanzi® and Nicogreen/Greenstar®. With approximately 4 million trees planted, Nicoter/Kanzi® is currently considered as one of the most successful club varieties of the past ten years. Since then Better3fruit has also commercialized Zari, Zonga, B3F44 and B3F45. Other apple and pear selections are in a pre-commercial testing phase with external commercial partners all around the world. Better3fruit's work does not stop with the breeding. We strongly believe in a guided market introduction in order to ensure that the fruit really finds its way to the consumer. For each variety, a different commercial strategy is developed, tailored to the variety. Better3fruit varieties are well-positioned in the market in order to generate ongoing added value for all the players in the fruit chain.



## **NECTARINE AND BRUGNONE CULTIVARS OBTAINED AT RESEARCH STATION FOR FRUIT GROWING CONSTANTA – ROMANIA**

**L.M. Dumitru\* and A. Indreias**

Research Station for Fruit Growing Constanta, Pepinierei Street, No.1, 907300 Valu lui Traian, Romania  
e-mail: [lianadumitru@yahoo.com](mailto:lianadumitru@yahoo.com)

The nectarine is the species with a good development under Romanian's conditions and the studies regarding its breeding and extension of new cultivars are achieved well. The objectives of nectarine and brugnone breeding were diversified as: quality of fruit, productivity of trees, ripening time, size, form, colour, flavor, sugar, taste and so many others characteristics and traits. This paper present these new nectarin cultivars, their biological performances and their future for extention in culture. All this varieties, like: 'Cora', 'Costin', 'Romamer 2', 'Marina', 'Liana', 'Valerica' and 'Anemona', improving the Romanian assortment.

## ASSESSMENT OF CHERRY CULTIVARS AND SELECTIONS IN LATVIA

**D. Feldmane\*, S. Ruisa, and I. Samsone**

Latvia State Institute of Fruit-Growing, Graudu Str.1,Dobele LV-3701, Latvia  
e-mail: daina.feldmane@lvai.lv

Sweet and sour cherry cultivars and selections were tested at Latvia State Institute of Fruit-Growing. The choice of sweet cherry cultivars is determined by our climate. Different cultivars (Estonian, Lithuanian, Russian) have been tested and several of them proved to be suitable for growing in Latvia. Besides the cultivar testing, sweet cherry breeding is continued with the aim to obtain winter-hardy and self-fertile sweet cherry cultivars with high fruit quality. As a result a new cultivar 'Paula' (winter-hardy, highly productive, large fruit size and good firmness) appropriate to our climate was developed. Perspective hybrids with improved properties (winter-hardy, large, firm fruits, good resistance to cracking) were selected. The first self-fertile hybrids have been bred also. Local and introduced sour cherry cultivars were evaluated for their productivity and fruit quality. The highest productivity was observed for the cultivar 'Bulatnikovskaya'. Comparatively high fruit weight was detected for the cultivars 'Tamaris' and 'Zentenes'. The fruits of the cultivar 'Desertnaya Morozovoi' had both high fruit weight and high total soluble solid content. The use of woodchip mulch and drip irrigation has been tested as well in order to determine the response of sour cherry cultivars on temporary drought stress and water supply. Significant rise of productivity was caused by drip irrigation for the cultivars yielding on the spurs.

## RESULTS OF POLLINATION STUDIES OF SOME NEW PLUM CULTIVARS IN LATVIA

I. Grāvīte<sup>1,2</sup> and E. Kaufmane<sup>1</sup>

<sup>1</sup>Latvia State Institute of Fruit Growing

<sup>2</sup>Latvia University of Agriculture

Graudu Str. 1, Dobeles, LV-3701, Latvia

e-mail: ilze.gravite@lvai.lv

One of the most important factors for the success of pollination and fertilization is the availability of suitable pollinator. Therefore the aim of study was to find the best pollinators in field condition for new plum cultivars 'Adele', 'Lotte' and 'Sonora', which were submitted in 2010 for registration in Latvia. Study was done at the Latvia State Institute of Fruit-Growing in 2008-2010. Cultivars, 'Experimentalfältets Sviskon', 'Jubileum', 'Julius', 'Minjona' and 'Victoria' were selected as pollinators. There were found significant differences among cultivars and pollination combinations. The results were significantly different also among the study years. The best pollination results for cultivar 'Adele' was found in combination with pollinator 'Victoria' (average fruit set % after June drop - 66.5%). Cultivar 'Lotte' showed the best results with cultivar 'Minjona' as pollinator (46.6%). Whereas cultivar 'Sonora' showed the best results of fruit set with cultivar 'Experimentalfältets Sviskon' as pollinator (53.9%). The most unsuitable pollinator in all crosses was the cultivar 'Julius'. Simultaneously open pollination experiment was established. Average fruit set in % after June drop for cultivar 'Adele' was 49.5, for cultivar 'Lotte' 37.7 and for cultivar 'Sonora' 53.3. Only cultivar 'Sonora' showed self-fertility (33.5%) in the self-pollination experiment. There were not significant differences in temperatures between years 2008 and 2009, but year 2009 was significantly drier. Spring of 2010 was very late, but sharp; during flowering time and time of fruit formation moisture was sufficient, but significantly higher was the average air temperature, relative humidity and humidity in the tree crown. Consequently, the results of pollination (average of all cultivars) in 2009 were 26.2% (fruit set from pollinations flowers) that differ significantly from 2008 (70.8%) and 2010 (36.2%).

## **SPC 207: A NEW BLUSH SWEET CHERRY FROM SUMMERLAND**

**C.R. Hampson\*, F. Kappel, R. MacDonald, and D.-L. McKenzie**

Agriculture and Agri-Food Canada  
Pacific Agri-Food Research Centre  
Summerland, B.C.  
Canada V0H 1Z0

SPC 207 is a large-fruited, self-fertile blushed cherry that ripens later than ‘Rainier’, ‘13N0770’ (Stardust™) or ‘Lapins’. The fruit is heart-shaped, with firm flesh, and medium to long stems. Crop levels are medium to high. Flavour is medium to good after storage and pitting is only slight. Bloom time of SPC 207 is after ‘Rainier’ but before Stardust™. The cultivar is being released to extend the harvest season for blush sweet cherries.

## **BEHAVIOUR OF SOME PLUM CULTIVARS IN THE CENTER OF TRANSYLVANIA**

**G. Roman, E. Hărșan\*, M. Mărghitaș, St. Wagner, A.P. Somsai, and M. Giurgiu**

Fruit Research Station Cluj, Romania, Horticultorilor Street 5, University of Agriculture Science and Veterinary Medicine, Cluj-Napoca, Romania

The plum is one of the most important fruit tree species in Romania due to the ecological plasticity and the high nutritional value of the fruits. At the Fruit Research Station of Cluj, 17 plum cultivars (14 new cultivars) were studied in order to introduce them in production. This cultivars has the following characteristics: early and average maturing of the fruits with high yield capacity (15 - 20 t/ha), large, beautifully coloured fruits, with crispy pulp. The following characteristics were evaluated: tree vigour, yields, time of flowering, time of fruit ripening and biochemical parameters of fruit quality. As a result of research carried out between 2004 - 2009, it is recommended to extend into production the cultivars President, Valor, Stanley, Minerva, Tuleu Gras, Silvia, Carpatin, Anna Spath, Black-Prince, with yields between 26 - 44 kg/tree, respectively 13.0 - 22 t/ha to the traditional cultivars Vânăț de Italia (10 kg/tree) and Vânăț Românesc (9 kg/tree).

## **RESULTS CONCERNING THE BEHAVIOUR OF SOME ROOTSTOCKS FOR PEACH TREE IN THE NURSERY AND ORCHARD RESEARCH TRIALS**

**A. Indreias\* and L.M. Dumitru**

Research Station for Fruit Growing Constanta, Pepinierei 1, 907300 Valu lui Traian, Romania

e-mail: indreiasalexandra@yahoo.com

The research carried out in Research Station for Fruit Growing Constanta, Romania, studied the behaviour in the nursery and orchard trials of five rootstocks obtain in breeding programme of peach rootstocks: 3 registered up to now Tomis 39, Tomis 28, T16 and two promising hybrids H.CT. 83.07.024 and H.CT. 83.07.005. In breeding works, the behaviour in research trials in the nursery and then in the orchard trials has a big importance for selection the best rootstocks for some cultivars. These rootstocks were grafted with 8 cultivars in the nursery field: 'Cora', 'ARK 134', 'Stark Delicious' and 'Anemona' (nectarine cultivars), 'Redhaven' and 'Raluca' (peach cultivars), 'Stark Saturn' and 'Filip'(peach cultivars with flat fruits). In the orchard we studied these rootstocks grafted with 'Redhaven' cultivar. The bred rootstocks were behaved very well in grafting process. They gave trees with different vigour, depending on rootstocks and cultivars and high yields of standard grafted trees ha<sup>-1</sup>. They present good compatibility with grafted cultivars, with no external deformations. Rootstock-scion combinations had a good behaviour in the orchard. All of these results show that studied peach rootstocks are good for peach and nectarine trees.

## **‘HONGARAM’: A RED FRUITED, HIGH AROMA TABLE GRAPE**

**J.H. Noh<sup>1\*</sup>, K.S. Park<sup>1</sup>, H.K. Yun<sup>2</sup>, Y.Y. Hur<sup>1</sup>, S.J. Park<sup>1</sup>, S.M. Jung<sup>1</sup>, and H.S. Hawang<sup>1</sup>**

<sup>1</sup>National Institute of Horticultural & Herbal Crop Science Institute, Suwon, South Korea

<sup>2</sup>Yeungnam University, Gyeongsan, South Korea  
e-mail: jeongho89@rda.go.kr

‘Hongaram’ is a new table grape cultivar, resulting from an interspecific (*Vitis* sp x *Vitis vinifera*) cross. It is diploid, late-ripening and red-fruited with high muscat aroma. Its mean date of bud burst, at Suwon, Korea is April 26, full bloom occurs on June 6, and fruit maturity on October 1. It has an excellent taste with abundant aroma and hard firmness. The mean weight of berries is 5.0 g, and mean total soluble solids concentration is 19.6° Brix. Although it is to cold resistant, ‘Hongaram’ is more suitable to a mild climate. It is a late-season table grape with high quality, comparable in taste, harvest date and cluster setting with ‘Campbell’s Early’. The harvest date is a major advantage in the Korean market, so it is recommended as an alternative to ‘Campbell’s Early’.

## PRODUCTIVE VALUE OF SOME POLISH SCAB-RESISTANT APPLE CULTIVARS GROWN ON DIFFERENT ROOTSTOCKS

M. Lewandowski<sup>1\*</sup>, E. Żurawicz<sup>1</sup>, and K. Rutkowski<sup>2</sup>

Research Institute of Horticulture, Konstytucji 3 Maja 1/3, 96-100 Skierniewice, Poland

<sup>1</sup>Fruit Breeding Department

<sup>2</sup>Storage and Processing Department

e-mail: Mariusz.Lewandowski@insad.pl

Resistance to apple scab is one of the most important objectives of the apple breeding program that has been conducted for many years at the Research Institute of Pomology and Floriculture (recently the Research Institute of Horticulture), Skierniewice, Poland. As a result of the breeding work three new apple genotypes resistant to apple scab have been released. Their names are: ‘Gold Milenium’, ‘Free Redstar’, ‘Melfree’. The first cultivar ripens in late summer, whereas two others are the autumn ripening apple genotypes. Results presenting their productive value were collected in the years 2004-2009 from the variety trial located in the Pomological Orchard at Skierniewice (Central Poland). Scab-resistant cultivars – ‘Retina’ (mid-early) and ‘Florina’ (late) were used as control. All cultivars were grown on four rootstocks: M.9, M.26, P14 and P60 and their trees were trained as a spindle tree. Pest control was applied as recommended for commercial apple orchards in Poland and fungicides against apple scab were not applied. During 5 seasons of investigation in the field the cultivars did not show any symptoms of apple scab and powdery mildew. Regardless of the rootstocks used the highest yield produced ‘Gold Milenium’ followed by ‘Melfree’, and the lowest yield was harvested from ‘Retina’ and ‘Free Redstar’. The genotypes also differed in fruit size. Irrespective of the rootstocks the smallest fruits had ‘Florina’ and the largest ‘Melfree’ and ‘Gold Milenium’, both grown on M.9 followed by ‘Retina’ and ‘Free Redstar’. In general all cultivars grown on P 60 produced smaller fruits than on other rootstocks involved in the experiment. Collected results indicate a high overall value of ‘Gold Milenium’. Its fruits are large, oblong conical, ground color is green-yellow with pink blush and internal fruit quality is good including high amount of soluble solids (Brix).



## NEW PEAR CULTIVARS FROM CZECH REPUBLIC

**F. Paprstein\*, A. Matejcek, and J. Sedlak**

Research and Breeding Institute of Pomology Holovousy Ltd, 508 01 Horice, Czech Republic  
e-mail: fp@vsuo.cz

There is a long tradition of pear growing on the territory of the Czech Republic. In recent years, the acreage of commercial pear plantations has been approximately 700 ha. At the end of the 20th century, a long term pear breeding programme was finalised. Twenty five pear cultivars were included into the National List of Plant Varieties as a result. Based on the evaluations made, the following cultivars were chosen for intensive production systems: Summer cultivars 'Isolda' and 'Radana', autumn cultivars 'Elektra', 'Karina', 'Morava' and 'Manon', early winter cultivars 'Vonka', 'David', 'Petra' and 'Dicolor', winter cultivars 'Bohemica', 'Astra', 'Dita' and 'Erika'. New plantations of cultivars 'Bohemica' (16.3 ha), 'Erika' (15.6 ha) and Dicolor (12.7 ha) were established in years 2007 - 2010. Concerning remaining cultivars, the acreage of newly established plantations is less than 5 ha. Important fruit and vegetative characters of cultivars recommended for commercial plantations were evaluated and are stated in the paper.

## RESULTS OF TEN-YEAR ROOTSTOCK TESTING FOR APPLE CULTIVAR RUBIN ON A FERTILE SOIL

**C. Piestrzeniewicz\*, A. Sadowski, and R. Dziuban**

Warsaw University of Life Sciences – SGGW, Department of Pomology,  
Nowoursynowska 159, 02-776 Warsaw, POLAND  
e-mail: cezary\_piestrzeniewicz@sggw.pl

The experiment was carried out on a fertile alluvial soil, at Warsaw-Wilanow, Central Poland, about 52°N, in the years 2001-2010. Nineteen very dwarfing and dwarfing rootstocks were tested for Rubin, a vigorous scion cultivar. Planting density was 2051, 2564 or 3077 trees per ha, depending on the expected tree vigour on different rootstocks. Ten years after planting the largest, according to the trunk cross-sectional area (TCSA), were trees on M.9 EMLA and on P 62. Significantly smaller were trees on Arm 18, then those on Unima and B 146. Even smaller were trees on M.27, but they did not differ significantly from those on P 16, P 66, P 65, No. 387, P 63, P 64, No. 280, P 22, J-TE-G, P 59 and PB-4. The smallest were trees on PJ 629; however, they did not differ significantly in size from those on PB-4 or P 59. The highest cumulative yield per tree was obtained on P 66, Arm 18, M.9 EMLA, B 491 and P 16. Significantly lower yields were obtained from trees on P 64, P 22, P 59, M.27, PB-4 or J-TE-G, and the lowest on PJ 629. The yield of trees on No. 280, Unima, P 65 and No. 387 was intermediate and did not differ significantly from that of trees on most rootstocks. Trees on P 59, PJ 629, PB-4, No. 280, J-TE-G, P 63, P 66, P 22, No. 387 and P 64 showed significantly higher yield efficiency (YE), expressed as a ratio of cumulative yield to the final TCSA, than those on M.9 EMLA or on P 62. Trees on the other rootstocks showed an intermediate YE. Mean fruit mass in the last three years (2008-2010) was slightly altered by rootstock. Significantly smaller were only fruits from the trees on PJ 629 and on PB-4 in 2008. Trees on P 63, B 491, P 16, P 66, P 65, P 64, P 22, No. 280, Arm 18, M.9 EMLA and Unima produced the highest cumulative yields per ha, whereas trees on PJ 629 the lowest.

## NEW SOUR CHERRY CULTIVARS FROM DRESDEN-PILLNITZ

**M. Schuster<sup>1\*</sup>, C. Grafe<sup>1</sup>, and B. Wolfram<sup>2</sup>**

<sup>1</sup>Institute for Breeding Research on Horticultural and Fruit Crops, Julius Kühn-Institute, Pillnitzer Platz 3a, D-02613 Dresden, Germany

<sup>2</sup>D-01257 Dresden, Guerickestraße 15, Germany  
e-mail: mirko.schuster@jki.bund.de

The focus in sour cherry breeding is directed on the fruit quality, the fertility, a high and stable fruit set and the resistance to biotic and abiotic stress. In the last years three cultivars, i.e. 'Jade', 'Achat' and 'Ruberllit' were realised. Two new sour cherry cultivars 'Coralin' and 'Spinell' and one further cultivar 'Jachim' with pillar growth habit are in realization since 2010. 'Coralin' shows good fruit characteristics and tolerance/resistance to leaf diseases caused by fungi like cherry leaf spot and shot hole. The cultivar 'Spinell' has large fruits with an excellent sweet sour cherry taste. The pillar sour cherry cultivar 'Jachim' is characterized by a natural distinctive upright growth habit with shorter internode length, narrow branch angles and a fruit set on short shoots. 'Jachim' show a good fruit set and fruit characteristics and tolerance to diseases.

## RESULTS OF SWEET CHERRY BREEDING IN DRESDEN-PILLNITZ

**M. Schuster<sup>1\*</sup>, C. Grafe<sup>1</sup>, and H. Schmidt<sup>2</sup>**

<sup>1</sup>Institute for Breeding Research on Horticultural and Fruit Crops, Julius Kühn-Institut, Pillnitzer Platz 3a, D-02613 Dresden, Germany

<sup>2</sup>Calvin Str. 16, D-35037 Marburg  
e-mail: mirko.schuster@jki.bund.de

Sweet cherry growing and breeding has a long tradition in Germany. After many historical changes in the past the sweet cherry breeding in Germany was started again in Dresden-Pillnitz in 2000. The main breeding goals are the fruit quality (size, firmness, taste, and colour), the high productivity, the self-compatibility and the resistance to biotic and abiotic stress. In result of the selection nine promising sweet cherry clones were evaluated: K8c,161, K8c,177, K8c,194, K8c,206 ('Krupnoplodnaja x 'Moldavska Tschernaja'), K21,376 ('Krupnoplodaja' × o.p.), F20,3,21 ('Büttners Rote Knorpelkirsche' × 'Lapins'), 90/4.0-8 ('Valeska' x 'Sunburst'), 92/31-22 ('Kordia' × 'Regina'). These clones are characterised by firm and large dark red fruits expect clone 90/4.0-8 with coloured fruits. The clones ripen in the time from the 4<sup>th</sup> to 6<sup>th</sup> cherry week ('Burlat' + 7 to +20 days). The clone Na 285 ('Knauffs' × 'St. Charmes') is an early ripening genotype with good fruit characteristics.

## AGROTECHNICAL AND BIOLOGICAL PROPERTIES OF SOME SOUR CHERRY CULTIVARS AND THEIR SUITABILITY FOR CULTIVATION IN CENTRAL POLAND

E. Szpadzik<sup>1</sup>, E. Jadczyk-Tobjasz<sup>1</sup>, and B. Łotocka<sup>2</sup>

Warsaw University of Life Sciences – SGGW, Poland

<sup>1</sup>Department of Pomology

<sup>2</sup>Department of Botany

Cultivars: ‘Schattenmorelle IR-2’ (British type of ‘Schattenmorelle’), ‘Koral’ (Poland), ‘Debreceni Bötermö’ (Hungary), ‘Újfehértói Fürtös’ (Hungary), ‘Karneol’ (Germany), and ‘Vowi’ (Germany) were examined for their biological and agronomical features in the years 2006-2009. ‘Debreceni Bötermö’, ‘Újfehértói Fürtös’, and ‘Karneol’ were the most vigorous (indicated by the increase of trunk – cross – sectional area: TCSA) compared to other tested cultivars. The high quality of pollen, investigated *in vitro* as viability and germination tests, and *in vivo* by germination capacity and pollen tube growth, was observed in ‘Koral’, ‘Schattenmorelle IR-2’, and ‘Vowi’, while the worse - in Hungarian cultivars, and ‘Karneol’. ‘Schattenmorelle IR-2’, ‘Koral’ and ‘Vowi’ had more fruits after self-pollination, compared with ‘Debreceni Bötermö’, ‘Újfehértói Fürtös’ and ‘Karneol’. Furthermore ‘Vowi’, ‘Schattenmorelle IR-2’, and ‘Koral’ gave the highest yield, whereas ‘Karneol’ and ‘Újfehértói Fürtös’ gave the lowest. It was revealed that Hungarian cultivars and ‘Karneol’ are partly self-fertile, while other tested cultivars had a high degree of autogamy and they set a lot of fruits after open pollination as well. It was found that pollen of ‘Schattenmorelle IR-2’ and ‘Koral’ increased fruit set significantly in Hungarian cultivars. Finally, ‘Debreceni Bötermö’ and ‘Újfehértói Fürtös’ had the highest fruit quality, evaluated by the mass (g), firmness (N), soluble solids content (%), acidity (after conversion into malic acid content), and ‘Vowi’ had the lowest.

## NEW APPLE CULTIVARS REGISTERED IN HUNGARY: ARTEMISZ, HESZTIA AND ROSMERTA

M. Tóth\*, S. Kovács, G. Ficzek, and M. Hevesi

Corvinus University of Budapest, Faculty of Horticultural Science, Department of Pomology, Villányi út 29-43, H-1118 Budapest, Hungary  
e-mail: magdolna.toth@uni-corvinus.hu

An apple breeding program has been carried out at the Department of Pomology since the beginning of the 90's. As a result of apple breeding for multiple resistance in 2011 three cultivars were registered in Hungary. The three new cultivars ('Artemisz', 'Hesztia', 'Rosmerta') are resistant against all the three most important apple diseases (apple scab, powdery mildew and fire blight). Scab resistance is controlled by the Vf gene originating from the species *Malus floribunda* 821 and transmitted by cultivar Prima. Heterozygote VfVf genotype of the cultivars was proved by molecular genetic examinations. *Artemisz*: Open-pollinated seedling of 'Prima'. Ripens in early or middle September. Fruit size is medium to large (140-200 g), shape is round conic. Suitable for processing (apple chips, juice and concentrate), as well as for fresh consumption after storage for those who like acidic apples. Its green ground colour is covered by an intense red or claret cover colour on the total fruit surface, gradually darkening with ripening, and this combines with dissolving bloom and rare but conspicuous white dots. Pedicel is short or medium long, pedicel hole is sometimes burned. Calyx and ovary are both closed. Its flesh keeps its outstanding firmness during storage. Flesh is light cream, juicy, taste is sweet-acid, aromatic. *Hesztia*: Free pollinated seedling of Prima. Ripens in early September, fruit size is medium, average weight is 180-250 grams, shape is slightly oblate, true conic. As a nice autumn apple, it is recommended for fresh consumption till December. Its yellowish white ground colour is overlaid on great part of fruit surface, the showiness of which is increased by a slight bloom and presence of small white lenticells. Pedicel is short or medium long, calyx is closed or half-open, ovary is totally closed. Flesh remains outstandingly firm during storage. Flesh colour is yellowish-white, taste is harmonic, combined with a tender aroma. *Rosmerta*: Hybrid of 'All Red Jonathan' and 'Prima'. Optimal harvest time is middle of September, fruits are rounded oblate, medium size, average weight is 140-150 grams. Resembling to Jonathan, it is recommended for fresh consumption and for processing as well. The yellowish green ground colour is covered by a bright red overlay on most of the fruit surface. Its cream coloured fruit flesh is medium firm. Pedicel is medium long, both calyx and ovary are closed. Its acidic sweet taste based on harmonic inner values and high polyphenol content is riched by a pleasant aroma. It has high level resistance to scab, moderate resistance to powdery mildew and fire blight.

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## NEW APPLE ROOTSTOCK SELECTIONS FROM ESTONIA

**T. Univer\***, **N. Univer**, and **K. Tiirmaa**

Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences, Polli Horticultural Research Centre, Karksi-Nuia 69108, Estonia  
e-mail: [toivo.univer@emu.ee](mailto:toivo.univer@emu.ee)

The Estonian breeding program was initiated in 1954, with the goal of obtaining apple rootstocks best suited for local soil and climatic conditions. The following parents were used: M.2, M.4, M.11, winter-hardy cultivars 'Anoka', 'Chulanovka', a seedling of 'Ranetka Purpurovaya' and local forms of *Malus prunifolia*. The program resulted in the release of 10 semi-vigorous and vigorous clones, marked with the letter E (for Estonia). A semi-vigorous rootstock E20 and vigorous rootstocks E53 and E56 were selected for production in nurseries. E-series rootstocks are easy to propagate in stoolbeds and their roots tolerate temperatures up to -14 - 16°C. The apple rootstock breeding program was continued in the years 1970-1985. E-series rootstocks and MM.106 have been used as donors. Finally, 43 clones were selected for testing in the orchard trials with cv. 'Pamyat Issayeva'. The most promising semi-dwarfing clones are 82-8-8, 82-8-10 and 82-8-19 (comparable to M.26) and dwarfing clones 82-8-12, 83-2-9 and 82-8-11 (comparable to B.9).

## **SD9238: A ‘REAL’ SEMI-DWARF GERMPLASM OF NECTARINE**

**Z. Wang\*, L. Niu, Z. Lu, G. Cui, and Y. Song**

Zhengzhou Fruit Research Institute, CAAS, Zhengzhou, Henan 450009, China  
e-mail: wang6399@126.com

Genetically controlled growth habit is a very important character affecting the peach tree's cultivation. Up to now, dwarf, compact type, columnar and weeping types have been reported. The SD9238, a new genetically controlled semi-dwarf type, was found in a breeding program which aimed at fruit size and flesh favor. It derived from the cross progeny of 25-10 × Mayfire which was made in 1992 in Zhengzhou Fruit Research Institute, China Academy of Agriculture Sciences. The parents 25-10 and Mayfire were normal nectarine selections. After several years' investigation, we confirmed that it was a new semi-dwarf mutant different from previously reported types because of its reduced stature, low bud break, shorter internodes and smaller tree size than standard peach tree. The genetic analysis showed that the semi-dwarf trait was controlled by a single dominant gene. The growth vigor of the hybrid is low. It has fewer lateral branches and the canopy has good light penetration. Before middle of May, SD9238's new shoots grew very slowly, even slower than dwarf type in this period. After that, it regained normal growth rate as standard type. Finally, the tree size was only 1/2 or 2/3 of the standard type. SD9238's shoot growth curve is a single 'S'. In a comparative test, SD9238 required no summer pruning and much less winter pruning. The fruits of SD9238 matured in the beginning of June and FDP (Fruit Development Period) was about 68 days. Its round, fully-covered red blush fruit, weighing around 135g, was very attractive. The flesh was white, melting and juicy. Its soluble solids content was about 12%, tasting sour. For commercial use, it still needed to be improved. But it's a promising germplasm for growth habits breeding of peach and nectarine.



## THE EFFECT OF 1-MCP AND CONTROLLED ATMOSPHERE ON THE QUALITY AND STORABILITY OF U 7979 AND 'MUTSU' APPLES

**K. Tomala\***, **K. Jeziorek**, **U. Ogłodzińska**, **T. Krupa**, **A. Sharafi**, and **M. Stepniowska**

Warsaw University of Life Sciences – SGGW, Faculty of Horticulture and Landscape Architecture, Department of Pomology, Warsaw, Poland

The investigation was carried out on U 7979 (apple clone resistant to scab bred by E. Pitera in the Department of Pomology, Warsaw University of Life Sciences; proposed name 'Chopin') and 'Mutsu' apples in the storage season of 2010/2011. The harvest date was determined on the basis of the induced ethylene method. Directly after harvest, some fruits were treated with 1-MCP at the concentration of  $0.64 \mu\text{l}\cdot\text{l}^{-1}$  for 24 h and the remaining apples were stored in the normal atmosphere (NA), at  $1^{\circ}\text{C}$ . Then fruits were placed under the ultimately assigned storage conditions. Three gaseous compositions of the controlled atmosphere (CA) were used ( $\text{CO}_2 : \text{O}_2$ ): 0.8:0.8; 1.2:1.2 and 2:2. Conditions of the common cold storage comprised the control. The effect of 1-MCP as well as conditions and duration of storage on the most important quality characteristics and fruit storability were determined after 4, 6 and 8 months of storage, both directly after storage and after 7 days of shelf life. Generally, among the apple cultivars resistant to scab, fruits of U 7979 clone stand out with a high firmness and titratable acidity, as well as high storability. In the common cold storage U 7979 and 'Mutsu' apples retained good quality up to 4 and 6 months after harvest, respectively. Longer storage under these conditions resulted in the excessive loss of flesh firmness and titratable acidity. The loss of firmness and of titratable acidity could be delayed to at least 8 months after harvest by storing apples in the CA, irrespective of their gaseous composition. Storage of apples in a common cold storage after subjecting them to 1-MCP allowed retaining apple quality not poorer than in those stored in the CA, but not treated with 1-MCP. However, the treatment of 'Mutsu' and U 7979 clone apples with SmartFresh (1-MCP) preparation for the 8-month storage period at CA conditions seems not to be justified.

## **GROWTH AND CROPPING OF 'JONAGORED' APPLE TREES DEPENDING ON ROOTSTOCK AND AUTUMN NITROGEN FERTILISATION**

**D. Wrona\* and W. Kowalczyk**

Department of Pomology, Warsaw University of Life Sciences – SGGW, Poland

In spring 1996 'Jonagored' apple trees on P 16 and P 22 rootstock were planted in Warsaw-Wilanów, on a fertile silty loam alluvial soil, spaced  $3.5 \times 1.2$  m. Herbicide strips were maintained along tree rows and sward in alleyways. Starting from autumn 2001, five fertiliser treatments were applied: (1) N-0 (check, without nitrogen); (2) N-50 ( $50 \text{ kg N ha}^{-1}$ , soil applied in autumn on the whole surface); (3) N - 100 ( $100 \text{ kg N ha}^{-1}$ , soil applied in autumn on the whole surface); (4) 5% urea (foliar nutrition in autumn); (5) N-50 + 5% urea ( $50 \text{ kg N ha}^{-1}$ , soil applied in autumn + 5% urea, foliar nutrition in autumn). Nitrogen was applied as urea, in autumn after harvest. Neither dose or mode of N fertilisation had any effect on the tree growth estimated by the trunk cross-sectional area after nine years (spring 2011) as well as its increase within nine years (autumn 2001 - spring 2011). No significant response to autumn nitrogen fertilization was noted on cumulative yield of apple trees for the years 2002-2010 and mean fruit weight. The cropping efficiency coefficient for nine years of bearing (2002-2010) calculated in relation to the final of the trunk cross-sectional area (spring 2011), did not depend on nitrogen fertilization either. Growth of trees, yield, fruit size and cropping efficiency coefficient depended on rootstock. Trees on P 22 showed a less vigorous growth, was lower yield per tree and smaller fruit in comparison with trees on P 16. However, the cropping efficiency coefficient was higher for trees on P 16 rootstock.

**Poster session**

**MANAGEMENT AND RESEARCH ON GENETIC  
RESOURCES (PART 1)**



## DEVELOPING A CORE COLLECTION OF OLIVE (*Olea europaea* L.) BASED ON MOLECULAR MARKERS (DArTs, SSRs, SNPs) AND AGRONOMIC TRAITS

A. Belaj<sup>1\*</sup>, M. del Carmen Dominguez-García<sup>1</sup>, S. G. Atienza<sup>2</sup>, N. M. Urdíroz<sup>3</sup>, R. De la Rosa<sup>1</sup>, Z. Satovic<sup>4</sup>, A. Martín<sup>2</sup>, A. Kilian<sup>5</sup>, I. Trujillo<sup>3</sup>, V. Valpuesta<sup>6</sup>, and C. Del Río<sup>1</sup>

<sup>1</sup>IFAPA CentroAlameda del Obispo. Avda. Menendez Pidal s/n 14080, Cordoba, Spain

<sup>2</sup>Departamento de Mejora Genética. Instituto de Agricultura Sostenible. Consejo Superior de Investigaciones Científicas Apdo. 4084, E-14080 Cordoba. Spain

<sup>3</sup>Departamento de Agronomía. E.T.S.I.A.M. Universidad de Córdoba. Edif. Celestino Mutis-C4. Campus Univ. de Rabanales. Carretera Madrid-Cádiz, Km. 396. 14071. Cordoba.Spain

<sup>4</sup>Faculty of Agriculture, University of Zagreb, Svetosimunska 25, 10000 Zagreb, Croatia

<sup>5</sup>DArT P/L, PO Box 7141 Yarralumla, Canberra, ACT 2600, Australia

<sup>6</sup>Departamento de Biología Molecular y Bioquímica. Facultad de Ciencias. Universidad de Málaga. Campus de Teatinos, s/n. 29071 Málaga  
e-mail: angjelina.belaj@juntadeandalucia.es

Agronomical traits, SSR, SNP and DArT molecular markers have been used in the world's largest germplasm collection (IFAPA, Centre Alameda del Obispo, Cordoba, Spain) to study: a) the patterns of genetic diversity and underlying genetic structure among 361 olive accessions as well to b) construct a set of core collections chosen to represent the bulk of the genetic diversity contained in this germplasm collection. Two different algorithms MSTRAT and PowerCore, based on M (maximization) strategy were used to build the core collections. Our results confirm that the germplasm collection is a useful source of genetically diverse material for researchers, olive breeders and growers. We also found that geographical origin is an important factor structuring genetic diversity in olive. Subsets of 18, 27, 36, 45 and 68 olive accessions, representing respectively 5%, 7.5%, 10%, 12.5% and 19% of the whole germplasm collection, were selected based on the information obtained by the whole data set as well as each marker type considered individually. Our results indicate that, the core collections that represent 19% and 10% of the whole collection size, could be considered as optimal. Due to its high efficiency at capturing all the alleles/traits states found in the whole collection the core size of 68 accessions could be of great interest for genetic conservation applications in olive. The high average genetic distance and diversity and the almost equal representation of accessions from different geographical regions, indicate that the core size of 36 accessions, would be the working collection for olive breeders.

## INVESTIGATION OF KOLOMIKTA KIWI (*Actinidia kolomikta*) PHENOTYPIC AND GENETIC DIVERSITY

L. Česonienė\* and R. Daubaras

Kaunas Botanical Garden of Vytautas Magnus University, Ž.E. Žilibero 6, Kaunas LT–46324, Lithuania

e-mail: laimac@hotmail.com

*Actinidia kolomikta* Maxim. has been gradually introduced into culture in Lithuania, along with successive assessment of its possibilities to adapt to the country's climatic conditions. *A. kolomikta* possesses exceptionally decorative properties and produces valuable berries. Therefore it may supplement the assortment of berry plants. The aim of this study was to distinguish the informative phenotypic characteristics and to evaluate genetic diversity of *A. kolomikta* germplasm collection at Kaunas Botanical Garden of the Vytautas Magnus University. The following traits were the most informative for phenotypic characterization of cultivars and clones: variegation intensity of leaves, size and distribution of flowers as well as berry size and shape. Female cultivars differed in the total number of fruiting shoots per meter length of two-year-old shoots. DNA investigations by RAPD method defined significant genetic diversity of *A. kolomikta* accessions and the level of their relationship. The percentage of polymorphism ranged from 55.6 to 80.0%. The highest genetic identity have been established for the female clones F2 and F4 ( $GD_{xy}=0.059$  and for the male clone M1 and female clone F2M2 ( $GD_{xy}=0.094$ ). Two specific markers were identified with the primers OPC-02 and 2B for the cultivar Laiba and the female clone F4M4. The dendrogram grouped the accessions by UPGMA method and revealed two main clusters. 'Laiba' proved to be the most divergent cultivar and joined to the other accessions at the 0.824 genetic distance.

## CRANBERRY BUSH *Viburnum opulus* – A NEW PLANT FOR HORTICULTURE

**R. Daubaras\* and L. Česonienė**

Kaunas Botanical Garden of Vytautas Magnus University, Ž.E. Žiliberio 6, Kaunas LT–46324, Lithuania

Interest in the biochemical composition of *Viburnum opulus* fruit has increased because of food industry demand for natural substances. To increase the use of *V. opulus* fruit in foods and pharmaceuticals, a better understanding of their biochemical components and health-promoting activities is needed. The aim of this study was to compare the biochemical components of *V. opulus* accessions and select the most valuable genotypes as sources of health-promoting components. This study corroborates the value of *V. opulus* as a potential source of biologically active substances. This research work was conducted to determine biochemical variability of *V. opulus* and to select the genotypes that could best serve as sources of health promoting substances. Twelve selected genotypes were evaluated. Fruits of the local clone P3 were characterised by large amounts of total phenolics, ascorbic acid, and reducing sugars. *V. opulus* var. *sargentii* and *V. opulus* var. *americanum* contained exceptionally large amounts of total phenolics, 1504.0 and 1455.0 mg/100 g, respectively. The amount of ascorbic acid varied from 12.4 to 44.4 mg/100 g, the amount of carotenoids varied from 1.4 to 2.8 mg/100g, the amount of anthocyanins varied from 23.2 to 49.9 mg/100 g, and the amount of total phenolics varied from 733.0 to 1504.0 mg/100 g.

## CONSERVATION AND USE OF FRUIT GENETIC RESOURCES IN LITHUANIA

**B. Gelvonauskis<sup>1\*</sup>, J. Labokas<sup>2</sup>, S. Žilinskaitė<sup>3</sup>, D. Gelvonauskienė<sup>4</sup>, and L. Česonienė<sup>5</sup>**

<sup>1</sup>Plant Gene Bank, Stoties 2, Akademija, Kedainiai distr. Lithuania

<sup>2</sup>Institute of Botany of Nature Research Centre, Žaliųjų Ežerų 49, Vilnius, Lithuania

<sup>3</sup>Vilnius University Botanical Garden, Kairėnų 43, Vilnius, Lithuania

<sup>4</sup>Institute of Horticulture of Lithuanian Centre of Agricultural and Forestry Sciences, Kauno 30, Babtai, Kaunas distr., Lithuania

<sup>5</sup>Kaunas Botanical Garden of Vytautas Magnus University, Ž.E. Žilibero 6, Kaunas, Lithuania

e-mail: b.gelvonauskis@agb.lt

In Lithuania, the genetic resources of fruit plants are mainly concentrated, preserved and studied in four institutions: the Institute of Horticulture of Lithuanian Centre of Agricultural and Forestry Sciences, the Institute of Botany of Nature Research Centre, the Vilnius University Botanical Garden and the Kaunas Botanical Garden of Vytautas Magnus University. Cultivars of apples, plums, sour and sweet cherries, black currants and strawberries are conserved at the Institute of Horticulture. There are 1470 accessions of fruit plants in the collections of the Institute. The most numerous is the apple collection with nearly 800 accessions. The genetic resources of fruit plants were used in different breeding programmes and 85 cultivars were bred. The genetic resources of raspberries, blackberries, wild strawberries, lingonberries, cranberries, blueberries, mostly of local origin, are maintained at the Institute of Botany as well as studied *in situ*, in their original habitats. Valuable forms of local origin and cultivars of edible honeysuckle, Japanese quince (*Chaenomeles*), raspberries and blackberries, grapes are maintained in the field collections at Vilnius University Botanical Garden. Those collections contain more than 750 accessions. Rich collections of snowballs, actinidia, cranberries, highbush blueberries and some others are established at Kaunas Botanical Garden. There are more than 300 cultivars and forms of local origin as well as forms introduced from other countries. The evaluation of accessions (contents of biologically active compounds, vitamins and other substances) of berries, tolerance to the local climatic conditions, technological aspects of propagation and cultivation and other properties are being studied.



## ESTABLISHMENT OF FIRST COLLECTION OF IRANIAN *Rubus* GERMPLASM AND PRELIMINARY STUDY OF GENETIC DIVERSITY, POMOLOGICAL POTENTIAL AND NUTRITIONAL VALUE OF THE ACCESSIONS

A. Gharaghani<sup>1\*</sup>, S. Eshghi<sup>1</sup>, S. Hossein Ali Momeni<sup>2</sup>, and Z. Keshavarz<sup>2</sup>

<sup>1</sup>Assistant professors of department of horticultural sciences, Shiraz University, Shiraz, Iran

<sup>2</sup>Master students of horticultural sciences, Shiraz University, Shiraz, Iran

*Rubus* is one of the most diverse genera in the plant kingdom with Approximately 740 species. *Rubus* fruit species are a rich source of anthocyanins and other polyphenolic antioxidants, so interest on them are increasing because of the possible beneficial implications in human health. Genetic resources of *Rubus* species in Iran are very diverse and because of many inter specific hybridization there is no agreement among botanists for the real number (4 - 10) of *Rubus* species in Iran. Based on literature review of botanical references, there are at least 4 main and distinct species of *Rubus* in Iran. Two of them (*R. anatolicus* and *R. caesius*) are widely distributed from wet climate in the north of Iran (caspien sea) to cold climate in the west and even some semiarid climates in the south west of the country. But other two species (*R. persicus* and *R. hyrcanus*) have limited distribution just in the wet climatic condition of the north of Iran. Except the botanical studies, there is no report on the *Rubus* genetic resources of Iran especially from the horticultural point of view. So we collected the representatives of this valuable germplasm from all around the country and established the first collection of Iranian *Rubus* species. We also started to study the genetic diversity, pomological potential and nutritional value of accession to select the promising individual for breeding purpose. Preliminary evaluation (mainly morphologic) showed that accessions of species are very diverse for morphological and fruit characters. Details of collecting and establishment of collection, characters and distribution of the main species and also the preliminary evaluation will be discussed.

## ESTABLISHMENT OF QUINCE (*Cydonia oblonga* Mill.) GERMPLASM COLLECTION FROM VARIOUS REGIONS OF IRAN

H. Abdollahi<sup>1</sup>, M. Alipour<sup>2</sup>, M. Khorramdelazad<sup>2\*</sup>, S. Mehrabipour<sup>2</sup>, A. Ghasemi<sup>3</sup>, M. Adli<sup>4</sup>, D. Atashkar<sup>1</sup>, and M. Akbari<sup>5</sup>

<sup>1</sup>Horticulture Department, Seed and Plant Improvement Research Institute, Karaj, Iran

<sup>2</sup>Faculty of Agriculture, Azad University, Science and Research Unit, Tehran, Iran

<sup>3</sup>Seed and Plant Section, Agriculture and Natural Research Center of Isfahan, Isfahan, Iran

<sup>4</sup>Seed and Plant Section, Agriculture and Natural Research Center of Khorasan, Mashad, Iran

<sup>5</sup>Faculty of Agriculture, University of Guilan, Rasht, Iran

Significant variation of quince genotypes and native cultivars is found at the temperate areas of Iran. For using this variation in the breeding programs, mainly for fire blight resistance, this research was performed with the aims of collecting and establishment of the first Iranian quince germplasm collection. More than 45 quinces selected from Central, North, North West and North East regions between 2004 and 2009, that all were differentiated by morphological and SSR markers, except two synonym genotypes, PK2 and SHA1. Both morphological and SSR markers clustered the genotypes to the separate groups based on the origin and typical characteristics of the fruits. The results also showed high variation in the fire blight resistance level of genotypes, so the most commercial fruits were observed among quince of central regions that usually expressed moderate to high level of fire blight susceptibility. The quinces of North West areas had lower rate of commerciality and higher tolerance to the fire blight, primarily evaluated in the conventional orchards of the native cultivars. The fire blight resistance also significantly affected by rootstocks, as most tested cultivars demonstrated higher susceptibility level on the hawthorn seedlings, compared to the quince seedling rootstocks. Quince genotypes of North with highest similarity to the wild ancestors demonstrated low fruit quality, late to very late fruit maturity and high fruit set. These genotypes also clustered as most dwarfing and showed the lowest level of leaf chlorosis in the calcareous soils.

## ANALYSIS OF SSR MARKER POLYMORPHISM AMONG KOREAN GRAPES

Y.-J. Oh<sup>1</sup>, J.-Y. Park<sup>1</sup>, I.-C. Son<sup>1,2</sup>, and D. Kim<sup>1\*</sup>

<sup>1</sup>Department of Horticultural Science, Chungbuk National University, Cheongju 361-763, Korea

<sup>2</sup>National Institute of Horticultural & Herbal Science, Suwon 440-760, Korea

In Korean table grape breeding program, just a few numbers of genetic sources including 'Campbell Early', 'Himrod Seedless' and 'Kyoho' were used for hybridization. The genetic distance between the Korean developed cultivars could be very close because of the narrowness of genetic background among parental materials. To get the basic information for extensive genetic diversity of future grape breeding, 10 SSR markers were analyzed among Korean grapes. A total of 25 grapes including 10 Korean released cultivars and 15 parental cultivars were analyzed. As a result, 120 alleles were detected within the range of 6 to 19 alleles. The polymorphism information content (PIC) ranged between 0.3235 (VVMD7) and 0.8724 (ssrVrZAG79), and the mean PIC was 0.7354. The genetic relationship was analyzed through the neighbor-joining cluster method, 25 grape cultivars were classified into 6 groups. The 1st group consisted of 'Heukbosuk', 'Suok', 'Bniizu', 'Kyoho', and 'Muscat Hamburg'. The 2nd group consisted of 'Moore Early', 'Thompson Seedless', 'Doonuri' and 'Schuyler'. The 3rd group included 'Hongdan', 'Hongisul', 'Tamnara', 'Campbell Early', 'Himrod Seedless', 'Jinok', 'Delaware', and 'Super Hamburg'. The 4th group consisted of 'Chungsu' and 'Seibell 9110'. The 5th group consisted of 'Heukgoosul', 'Golden Muscat', 'Pione', 'Tano Red', and 'Tankeumchu'. The 6th group included just a 'Centennial' grape. Tested 10 SSR markers could classify the 7 out of 10 Korean cultivars following their parentage depending on genetic distance. In addition, analyzed 9 Korean grapes except 'Doonuri' could be identified with the profiling of 4 SSR markers including VVMD27, ssrVrZAG6, VMC6E1, and VMC6G1.

## MORPHOLOGICAL CHARACTERISTICS AND GENETIC RELATIONSHIPS AMONG KOREAN TABLE GRAPE CULTIVARS

Y.-J. Oh<sup>1</sup>, J.-Y. Park<sup>1</sup>, S.-I. Oh<sup>1</sup>, J.H. Park<sup>1</sup>, I.-C. Son<sup>1,2</sup>, J.H. Noh<sup>2</sup>, and D. Kim<sup>1\*</sup>

<sup>1</sup>Department of Horticultural Science, Chungbuk National University, Cheongju 361-763, Korea

<sup>2</sup>National Institute of Horticultural & Herbal Science, Suwon 440-760, Korea

To offer the information for protecting and discriminating of Korean released grapes, genetic relationships among Korean grapes and their parents were analyzed through morphological investigation. Morphological characteristics were investigated among 25 grape cultivars included 10 Korean bred cultivars and 15 parental cultivars according to UPOV test guideline for grapevine. The investigated morphological characteristics of the 25 grape cultivars were coded according to given values, and were analyzed through the unweighted pair group method with arithmetic (UPGMA) method using the NTSYS program. Analyzed 25 cultivars were divided into 4 groups based on similarity of 0.248. The 1st group included 'Moore Early', 'Hongisul', 'Delaware', 'Tamnara', 'Schuyler', 'Heukgoosul', and 'Himrod Seedless'. The 2nd group consisted of 8 cultivars including 'Campbell Early', 'Hongdan', 'Jinok', 'Super Hamburg', 'Chungsu', 'Tankeumchu', 'Seibel 9110', and 'Tano Red'. The 3rd group included 'Golden Muscat', 'Heukbosuk', 'Beniizu', 'Pione', 'Kyoho', 'Suok', 'Centenial' and 'Muscat Hamburg'. The 4th group contained just a 'Thompson Seedless'. According to the results of cultivar classification based on morphological characteristics, one or more parent cultivars were mixed within 3 groups. Unfortunately, we could not confirm any closer relationship between Korean grapes and each of their parental cultivars. It could be a result of morphological discrimination takes a long time until the expression of specific characteristics, and morphological similarity is also affected by environments, nutritive conditions and ecotype during the period.

## YIELDING CHARACTERISTICS OF *Rosa* TAXA

S. Kovács<sup>1\*</sup>, É. Szabóné Erdélyi<sup>2</sup>, D. Szilágyi<sup>1</sup>, and M. Tóth<sup>1</sup>

Corvinus University of Budapest, Faculty of Horticultural Science

<sup>1</sup>Department of Pomology

<sup>2</sup>Department of Mathematics and Informatics

Dogrose breeding for fruit purpose has been performed at Corvinus University of Budapest, Faculty of Horticultural Science, Department of Pomology. Our work is based on the extremely rich *Rosa* genetic material of Hungary. In the course of our investigations perspective genotypes have been raised from *R. zalana*, *R. indora*, *R. corymbifera*, *R. rubiginosa* and *R. canina*. Characteristics of flowering, yielding and growth properties of raised taxa have been evaluated, chemical content of the rosehips have been measured during ripening. With these information suitability of the investigated taxa for commercial orchards can be determined. For testing yielding characteristics shoots and twigs of different ages of *Rosa* taxa has been labelled. The number of flowers and flower clusters, later fruit set were recorded. Picking time have been determined according to the ripening time of the given taxon. The physical parameters (height, diameter, pedicel length, mass, number of seeds) of rosehips were measured in Fruit Analytics Laboratory of Department of Pomology. The aim of our investigations were to clarify whether there is a difference between physical parameters of fruits developed on different aged bearing woods. Single fruits and rosehips of bunches were evaluated separately. Our investigations showed that the fruit size depends on the age of bearing woods. In most cases the best fruits bear on 1 - 3 years old woods. Aging of bearing woods are the most conspicuous in *R. canina* and varieties of *R. canina*, *R. inodora*, and *R. corymbifera*, because fruit size reduction has been observed in 3-year-old bearing woods already. There is a very little statistical difference detected in physical parameters of fruits among 1-3 years old bearing woods of *R. Rubiginosa* as well as among 1-5 years old woods in *R. zalana*. Minimal differences were observed in physical parameters between single fruits and fruit groups. The statistical analysis only proved in *R. inodora*, that the more fruits grow in groups, the smaller is the fruit size.

## **A HISTOLOGICAL STUDY ON FRUIT SKIN AND ANTHER OF PLUM 'SOLDAM', APRICOT 'HARCOT' AND PLUMCOT (PLUM × APRICOT) 'HARMONY'**

**E.Y. Nam\*, S.K. Yun, J.H. Jun, I.K. Yoon, Y.U. Shin, and K.H. Chung**

Fruit Research Division, National Institute of Horticultural & Herbal Science, Rural Development Administration, Suwon 440-706, Korea  
e-mail: [eynam@korea.kr](mailto:eynam@korea.kr)

Hybrids between plums (*Prunus salicina* Lindl., *P. cerasifera* Ehrh. or their hybrids) and apricots (*P. armeniaca* L.) are referred to as plumcots. Interspecific crossing between plum and apricot has been conducted since 1997 to breed new cultivars at the National Institute of Horticultural & Herbal Science. Recently, 2 plumcot cultivars 'Harmony' and 'Tiffany' have been released. We have been studied on fruit appearance, quality, phenology, histological morphology of fruit skin and anther with plumcot 'Harmony', plum 'Soldam' and apricot 'Harcot', its cross parents. Blooming date and ripening time are about halfway between plum and apricot. Histological characteristics of fruit skin such as the number of cells, cell size, a distribution of hair and stomata have also a tendency about halfway between plum and apricot. The anthers of plumcot 'Harmony' have no pollens. 'Harmony' should be planted with other apricot cultivars having same blooming time and should be needed artificial pollination.

## EVALUATION OF CANADIAN ACCESSIONS OF *Fragaria virginiana* MILLER WITH REGARD TO STRAWBERRY BREEDING

A. Ludwig<sup>1,2</sup>, M. Bartho<sup>3</sup>, S. Kuss<sup>4</sup>, C. Kuss<sup>4</sup>, S. Plaschil<sup>5</sup>, K. Olbricht<sup>1\*</sup>, and P. Scheewe<sup>3\*</sup>

<sup>1</sup>Hansabred GmbH & Co. KG, Radeburger Landstraße 12, 01108 Dresden, Germany

<sup>2</sup>Humboldt University of Berlin, Faculty for Agriculture and Horticulture, Department of Horticultural Plant Systems, Lentzeallee 75, 14195 Berlin, Germany

<sup>3</sup>Dresden University of Applied Sciences, Faculty of Agriculture and Landscape Management, Pillnitzer Platz 2, 01326 Dresden, Germany

<sup>4</sup>1836 Place Beauchesne, Montreal, QC, H1L3G9, Canada

<sup>5</sup>Bötzowstraße 45, 10407 Berlin, Germany

e-mail: k.olbricht@hansabred.org, scheewe@virentis.de

*Fragaria virginiana* Miller as well as *Fragaria chiloensis* (L.) Miller are the ancestors of *Fragaria* × *ananassa* Duch. Nowadays, innovative strawberry breeding programmes focus on the backcrossing of strawberry cultivars with its relatives. The introgression of accessions of wild species are valuable for cultivar improvement because of proven tunnel effects of aroma patterns and resistances in the breeding history of *Fragaria* × *ananassa*. In this contribution, seeds from five Canadian sources were sown. The resulting plants were cultivated and taxonomically described as *F. virginiana* ssp. *platypetala*, *F. virginiana* ssp. *virginiana* and the nothomorph form *F. virginiana* ssp. *glauca* × *F. virginiana* ssp. *platypetala*. The plants were described regarding to growth habit, resistances, flower characteristics including sexes, earliness of fruit ripening, fruit shape, size, colour, flavour, yield, Brix value and day neutrality. The selection of individuals resulted in a group of potential breeding partners with promising parameters, in particular day neutrality. These plants have been added to the Hansabred germplasm collection. Today, they are already used for breeding of new cultivars.

## RESCUE OF OLD SWEET CHERRY CULTIVARS

**F. Paprstein<sup>1\*</sup>, J. Sedlak<sup>1</sup>, and V. Holubec<sup>2</sup>**

<sup>1</sup>Research and Breeding Institute of Pomology Holovousy Ltd, 508 01 Horice, Czech Republic

<sup>2</sup>Crop Research Institute, Drnovska 507, 161 05 Praha-Ruzyne, Czech Republic  
e-mail: fp@vsuo.cz

The area of the present Czech Republic was rich in fruit crops in the last centuries. A considerable amount of cultivars had been cultivated until the beginning of the 20th century. Since the half of the last century, there has been a continuing destruction of old plantations. This destruction is connected with the intensification of agricultural production. Since 1994, the programme for collecting and long term conservation of fruit landraces at the territory of the Czech Republic has been carried out. Collecting expeditions were organised in the areas not affected by the intensive agricultural production and recreational expansion. Expert activities were organized at the time of fruit ripening. Determination of cultivars was carried out together with description of health status, short characterization and localization of the tree. Found and original cultivars and forms of grown sweet cherries represent a valuable material for breeders as donors of requested characteristics (for example resistance against diseases pests and abiotic stresses). Sweet cherry landraces have also very good adaptability in areas of their distribution and growing. There are local cultivars of sweet cherry, which are distributed and typical for certain areas 'Chlumecka', 'Techlovicka', 'Pivovka' ('Sychrovska chrupka'), 'Pumra'. Trees of mazzard *Prunus avium* were found at an altitude of 800 – 1 000 m (Giant Mountains), they were in a very good health condition and their age was estimated to be more than 100 years. Surprisingly, it was the fruit tree species found at the highest altitude. Descriptions of important sweet cherry landraces are stated in the paper.



## **PREDICTIVE MODELING OF SPECIES DISTRIBUTIONS: OCCURRENCES AND ENVIRONMENTAL VARIABLES WITH FRUIT TREES**

**M.P. Errea<sup>1</sup>, A. Pina<sup>2\*</sup>, M.T. Espiau<sup>2</sup>, and P. Errea<sup>2</sup>**

<sup>1</sup>Instituto Pirenaico de Ecología (IPE), Avda de Montañana 1005, Campus de Aula Dei, 13034, Zaragoza, Spain

<sup>2</sup>Unidad de Fruticultura, CITA de Aragon, Avda de Montañana 930, 50059, Zaragoza, Spain

The identification of what geographic and environmental factor might determine species range limits in fruit trees is specially important for understanding why some regions, such a mountains, have elevated biodiversity. The main goal of this work was to predict the spatial distribution of old pear and apple cultivars located in abandoned areas and at risk of extinction, that are supposed to have an important agronomic and genetic value. This study was conducted in mountain areas of Aragon (Spain) with different geographic features. These areas included the Pyrenees and Iberian System. Locality data for these local species were collected using a GPS and an intensive inventory of these geographic areas was elaborated with an important number of local pear and apple trees. Based on this inventory, a specialized database was created to describe each tree's individual attributes, including different environmental variables (climate and topography) that influence in the persistence of species and germplasm diversity. Thus, occurrence data from these regions combined with digital layers of environmental variables were used to predict potential distributions of these fruit trees in both geographic areas using a niche-based model constructed from presence only data (Maxent). The study showed that Maxent model is improved if the analysis is performed in the mountain areas separately, optimizing the geographical area although the number of occurrences used is less. In addition, this model revealed that the influence of several environmental variables depend on the study geographic region, Mediterranean or Subalpine mountain climate. These results support our previous results obtained with multinomial logistic regression analysis frequently used for modelling species distributions. The environmental factors characterizing the potential geographic distribution of these fruit trees might help in future breeding programs to create new fruit tree crosses and varieties adapted to determined cultivated regions.

## **PLANT AND FRUIT CHARACTERISTICS OF *Arbutus unedo* L. AND *Arbutus andrachnae* L. FROM THE HIGHLANDS OF NORTHWESTERN TURKEY**

**N. Ekinci<sup>1</sup>, A. Sakaldas<sup>2</sup>, M. Seker<sup>3\*</sup>, and H. Ekinci<sup>4</sup>**

<sup>1</sup>University of Çanakkale Onsekiz Mart, Lapseki Vocational College, Lapseki, Çanakkale, Turkey

<sup>2</sup>University of Çanakkale Onsekiz Mart, Faculty of Agriculture, Department of Horticulture, 17020, Çanakkale, Turkey

<sup>3</sup>University of Çanakkale Onsekiz Mart, Faculty of Agriculture , Department of Horticulture, 17020, Çanakkale, Turkey

<sup>4</sup>University of Çanakkale Onsekiz Mart, Faculty of Agriculture, Department of Soil Science, 17020, Çanakkale, Turkey

The genus *Arbutus* (fam: Ericaceae) is represented by two species, *A. unedo* L. and *A. andrachnae* L., in the flora of northwestern of Turkey. Çanakkale region located in the northwestern Turkey is one of the main spreading locations for *Arbutus* species. The main objective of the present study is to present some phenological, plant and fruit characteristics of the *Arbutus* population. During the study, flowering time, average canopy size, tree height, trunk diameter and fruit yield were determined. Besides of plant characteristics some chemical properties including pH, soluble solid content (TSS), titrable acidity (TA), ascorbic acid, anthocyanins and minerals were determined in fully matured *Arbutus unedo* L. and *Arbutus andrachne* L. fruits collected from different sites of Çanakkale-Turkey. Furthermore preliminary results of strawberry tree fruit storage were presented. In view of the plant characteristics and chemical composition, cultivation of the species is also recommended.

## GENETIC DIVERSITY IN AN ALMOND GERMPLASM COLLECTION: APPLICATION OF A CHEMOMETRIC APPROACH

**O. Kodad<sup>1</sup>, G. Estopañán<sup>2</sup>, T. Juan<sup>2</sup>, and R. Socias i Company<sup>1\*</sup>**

<sup>1</sup>Unidad de Fruticultura, CITA de Aragón, Apartado 727, Zaragoza, Spain

<sup>2</sup>Unidad de Calidad y Seguridad Alimentaria, CITA de Aragón, Apartado 727, Zaragoza, Spain

Genetic diversity of the international almond (*Prunus amygdalus* Batsch) collection situated at the CITA, Zaragoza, Spain, was characterized by applying a chemometric approach. Oil and the major fatty acids were determined in 77 almond cultivars from 12 countries. The results revealed high genetic variability of the studied traits among the cultivars. Clustering of the cultivars from similar countries would suggest the existence of different almond populations. The present results did not show any clear separation of cultivars according to their geographical origin. The chemical composition of almond kernel could be used to study the genetic diversity of almond cultivars, but not to study its evolution around the world. The highest values of oleic and lowest of linoleic acids were determined in ‘Bonifacio’ and ‘Mollese’ from Italy, ‘Yosemite’ from USA, and ‘Pestañeta Menuda’ from Spain. These cultivars could be incorporated into almond breeding program to increase kernel quality.



**Poster session**

**GENETIC AND GENOMIC STUDIES OF FRUIT CROP  
PHYSIOLOGY**



## DETECTION OF PEAR S-ALLELES BY SETTING UP A REVISED IDENTIFIED SYSTEMS

F. Babaei<sup>1\*</sup>, H. Abdollahi<sup>2</sup>, and M. Khorramdelazad<sup>1</sup>

<sup>1</sup>Faculty of Agriculture, Azad University, Science and Research Unit, Tehran, Iran

<sup>2</sup>Horticulture Department, Seed and Plant Improvement Research Institute, Karaj, Iran

Most pear cultivars are self-incompatible, thus for proper and adequate fruit set need to the pollinizer trees with suitable compatibility of blooming period and alleles. This research was carried out in order to set up an affordable and reliable system to identify the self-incompatibility alleles in pear cultivars of Iranian National Pear Collection. Partial or complete sequences of more than 200 pear S-alleles have been obtained from NCBI and aligned by ClustalW2. Since ClustalW2 can not carry out aligning a large proportion of data, similarity of sequences were compared in Microsoft Word and were clustered in distinct groups of similarity. By this way, population of self-incompatibility alleles abbreviate in distinguishable 64 S-alleles. Also, 5 conserved regions of each alleles, C1, C2, C3, RC4 and C5, were revealed thoroughly and selected two regions for designing one primer pair, FB-F/FB-R2, with the ability of extreme amplification. The restriction map of amplified regions by using most common cleavage enzymes, *DraI*, *EcoRI*, *MspI* and *HaeIII*, was obtained by OLIGO software for complete separation of S-alleles. The results of revised grouping showed homonym group of *S4*, also synonym alleles such as *S22* and *S3* in NCBI. Detection of pear S-alleles in collection demonstrated frequencies of 21, 10.5, 13.1 and 7.8 percent for *S1*, *S2*, *S4* and *S5* alleles, with the same frequency of S-alleles in European cultivars. The results showed adequate precision, simplicity and affordability of PCR-RFLP for indentifying of S-alleles in pear cultivars.

## **PERFORMANCE OF TREE GROWTH CHARACTERISTICS IN SELECTED PROGENIES OF COLUMNAR APPLE CULTIVARS**

**J. Blažek**

Research and Breeding Institute of Pomology Holovousy Ltd., 508 01 Horice, Czech Republic  
e-mail: blazek@vsuo.cz

The growth habit of columnar apple trees was assessed during 2006-2010 in 11 progenies preliminary selected from 7 different parental combinations. Progenies of the same parental combination involved either trees of different age and trees on different rootstocks (M.9 and seedling rootstocks). In total 308 trees were assessed. Particular progenies were mutually significantly different both in their mean tree vigour defined by their final tree height, and by mean length of annual terminal prolongation as well as by variability these characteristics within progenies. In some progenies the length of annual terminal prolongation was significantly influenced by their current fruit set level. Particular progenies were furthermore significantly distinguished in mean number of side shoots and especially in their general variability. The highest numbers of ramification were generated by genotypes on seed rootstocks and also by genotypes that in a given year had no fruit set. Smaller differences among progenies were found in intensity of central axis by spurring (their number and length) and in the extent of its bare wooding. Application of seed rootstocks significantly prolonged age when trees of studied genotypes reached their first yielding. Significantly lower productivity presented the most vigorous trees growing on the seedling rootstocks. This effect of tree vigour had not been observed in any progeny on rootstock M.9. Certain difference in extent of biennial bearing tendency among evaluated progenies seems to be also relevant.



## INFLUENCE OF PLUM ROOTSTOCKS ON THE DYNAMIC OF DRY MATTER IN THE ANNUAL SHOOTS OF CULTIVAR 'VICTORIA'

D. Dekena<sup>1\*</sup>, I. Alsina<sup>1</sup>, and J. Lepsis<sup>2</sup>

<sup>1</sup>Latvia University of Agriculture, 2 Liela street, Jelgava, LV – 3001, Latvia

<sup>2</sup>Pure Horticultural Research Centre Pure, 2 Abavas street, Tukums reg., LV – 3124, Latvia

One of the most important preconditions for establishment of high yielding and sustainable orchards is the choice of rootstock. Therefore the question about rootstock influence on plum tree winterhardiness is actual. Dry matter dynamic during wintering period in annual shoots of plum trees was evaluated as factor influenced wintering. Investigations on *Prunus domestica* plum cv. 'Victoria' (2n = 48) were performed during wintering periods of 2008/2009, 2009/2010 and 2010/2011 in Püre HRC, Latvia, in orchard planted in 2001. There were used 16 rootstocks – 8 vegetative propagated St. Julien A, Brompton., Ackermann, Pixy, GF8/1, G5/22, GF655/2, Hamyra and 8 generatively propagated St Julien INRA2, St. Julien d'Orleans, St. Julien Noir, Brompton, Wangenheims Zwetche, St.Julien Wädenswill, Myrobolana, *Pr. cerasifera* var *divaricata*. Samples were taken 5 times per wintering period and dry matter content (mg g<sup>-1</sup>) was detected in laboratory. Fluctuations of dry matter content during 3 wintering periods show significant increase of dry matter during December and January. No significant effect of rootstock was stated. The less fluctuations in dry matter content of trees grafted on *Prunus cerasifera* (495 – 507 mg g<sup>-1</sup>) and 'Pixy' (503 – 516 mg g<sup>-1</sup>) were detected. It leads to assumption that these rootstocks are suitable for unstable weather conditions. The most fluctuating content of dry matter in one annual shoots was observed for trees grafted on 'Myrobolana' (496 – 541 mg g<sup>-1</sup>). The dynamic of dry matter shows differences in physiological processes during different weather conditions.

## IDENTIFICATION OF SELF-INCOMPATIBILITY ALLELES IN IRANIAN PEAR CULTIVARS USING PCR ANALYSIS

**M. Bagheri and A. Ershadi\***

Department of Horticulture, Faculty of Agriculture, Bu-Ali Sina University, Hamedan, Iran

In the present study the *S*-alleles of eighteen pear cultivars, thirteen cultivars planted commercially in Iran and five control cultivars, were determined. Using nine allele-specific primers designed for amplification of S1/S2, S5, S6, S7, S8, S9, S11, S12 and S14; as well as consensus primers, MPyC1F and MPyC5R 36 out of 38 *S*-alleles were detected. S4, S1 and S5 were the most common *S*-alleles that observed in eight, seven and five cultivars, respectively. On the basis of the length estimated and sequence alignment of PCR products two probably new alleles were detected in 'Domkaj' and 'Kaftar Bache' cultivars, which provisionally labeled S<sub>x</sub> and S<sub>y</sub>. For 17 cultivars both alleles [ 'Bartlett' (S<sub>1</sub>S<sub>2</sub>), 'Beurre Giffard' (S<sub>1</sub>S<sub>6</sub>), 'Comice' (S<sub>4</sub>S<sub>5</sub>), 'Doshes' (S<sub>4</sub>S<sub>7</sub>), 'El Dorado' (S<sub>1</sub>S<sub>7</sub>), 'Koshia' (S<sub>4</sub>S<sub>8</sub>), 'Paskolmar' (S<sub>1</sub>S<sub>5</sub>), 'Felestini' (S<sub>1</sub>S<sub>7</sub>), 'Domkaj' (S<sub>4</sub>S<sub>x</sub>), 'Ghousi' (S<sub>4</sub>S<sub>7</sub>), 'Kaftar Bache' (S<sub>4</sub>S<sub>y</sub>), 'Konjoni' (S<sub>4</sub>S<sub>8</sub>), 'Laleh' (S<sub>5</sub>S<sub>8</sub>), 'Sebri' (S<sub>1</sub>S<sub>4</sub>), 'Se Fasleh' (S<sub>1</sub>S<sub>5</sub>) and 'Starkrimson'(S<sub>1</sub>S<sub>8</sub>)] and two cultivars only one allele ['Pighambari' (S<sub>5</sub>) and 'Shah Miveh Esfahan' (S<sub>7</sub>)] were identified. Allele-specific PCR amplification was an efficient and rapid method to identify *S*-genotype of Iranian pear cultivars.

## MONOGENIC SIGN CONTROL OF A PEAR COMPACT HABIT

V.S. Girichev

All-Russian Horticultural Institute for Breeding, Agrotechnology, and Nursery, Zagorievskaya str, 4, Moscow 115598, Russia

The derivatives 'Nain Vert' with a monogenic sign of dwarfism represent the particular interest in pear breeding for compact type of crown. When used in hybridization as one of the parents derivatives of 'Nain Vert' in the heterozygous state, the segregation of dwarfism was observed within 50% of received progeny. The cultivars used in breeding program as a maternal form, such as 'Lada', 'Eseninskaja', 'Chigovskaja', 'Rovesnica', 'Belorusskaja Posdnjaya', 'Bananovaja', 'Petrovskaja', 'Vidnaja', and interspecific pear hybrid 03 had the segregation scheme in progeny 1:1 (dwarfs: not dwarfs). High output of compact seedlings in the progeny originating from hybrid (F<sub>2</sub> from 'Nain Vert') allows us to suggest that the compactness of this form is defined with one dominant gene in the heterozygous state. To test this hypothesis, we analysed the progeny of nine families using  $\chi^2$  technique. The segregation test showed that the actual values of  $\chi^2$ , equal 0,1 to 1,9 were less than the tabulated values (15,51). It means that the difference of empirical segregation from the theoretical prediction is not true. It indicates the presence of key gene (D) in the heterozygous state at donor D (F<sub>2</sub> from 'Nain Vert'). The compactness signs of pear seedlings appear in the first year of life that allows to select the seedlings before planting them in the orchard.

## **THE EFFECT OF IBA, NAA HORMONES ON ROOTING OF RUSSIAN OLIVE (*Elaeagnus angustifolia*) CUTTING**

**V. Jajarmi**

Islamic Azad University Bojnord Branch, Iran  
e-mail: Vahid\_jajarmii@yahoo.com

To study the effect of IndoleButyric Acid (IBA) and Naphthalene Acetic Acid (NAA) on rooting, branch cutting of russian (Shekaei, Anabi variety), an experiment was carried out with three replications, through factorial method in completely randomized design at green house. The hormones for IBA was: 0 (distilled water), 1000, 3000, 6000 mgr/lit and for NAA, 2000, 3000, 4000. The considered cutting were put in solutions for 60, 90 seconds and right after that they were put in three culture media of grit, sand, soil with decomposed manure. The longest root obtains of IBA 3000 mgr/lit + 3000 mgr/lit of NAA in sand bed. The highest drought weight resulted for IBA 3000 mgr/lit + 2000 mgr/lit NAA in sand media culture with put cutting in solution for 90 second. The best root production percent was taken from 6000 mgr/lit IBA and 90 seconds in sand culture. The highest root number was related to IBA 4000 mgr/lit. The best media culture was sand. The number of root, root long decrease in 6000 mgr/lit IBA.

## ISOLATION AND EXPRESSION ANALYSIS OF GIBBERELLIN 20-OXIDASE GENE IN DWARF ROOTSTOCK OF PEAR ‘ZHONGAI 1’

S. Jiang<sup>1,2</sup> L. Xuan<sup>1,2</sup>, C. Ou<sup>1,2</sup>, F. Wang<sup>1,2</sup>, Z. Wang<sup>1,2</sup>, F. Cheng<sup>1,2</sup>, and Z. Li

<sup>1</sup>Research Institute of Pomology, Chinese Academy of Agricultural Sciences, Xingcheng 125100, Liaoning

<sup>2</sup>Key Laboratory of Fruit Germplasm Resources Utilization, Ministry of Agriculture, Xingcheng 125100, Liaoning

<sup>3</sup>Forestry Administration of Daxing, Daxing Beijing 102600 China Forestry Administration of Daxing, Beijing 100076

Gibberellin (GA) 20-oxidase gene was isolated from the young leaves of ‘Zhongai 1’ by reverse transcription polymerase chain reaction (RT-PCR) and rapid-amplification of cDNA end (RACE). The full-length cDNA consists of 1179 nucleotides and encodes a putative protein of 392 amino acids residues. The predicted protein has all conserved functional active sites and typical unit as well as the GA20-oxidase family signature. Sequence alignment and phylogenetic analysis revealed that the predicted protein sequence shared 91.58% identity with apple’s. So we confirmed the gene was the GA20-oxidase gene of ‘Zhongai 1’, and named it as *PyGA20ox1* (GenBank accession numbers was HQ833589). The semi-quantitative RT-PCR analysis results of this gene in three pear cultivars showed that the expression intensity of *PyGA20ox1* in leaves of three pear cultivars had the same trend as ‘Zhongai 1’ < ‘JinXiang’ < ‘ZaoSu’ in all tested growing periods. This is conformed with the plant height and indicated that the expression intensity of *PyGA20ox1* was related with plant height.

## **IDENTIFICATION OF GENES THAT VARY THEIR EXPRESSION DURING BUD DORMANCY RELEASE IN TEN PEACH VARIETIES HAVING LOW AND MEDIUM CHILLING REQUIREMENTS**

**C. Leida\*, M.L. Badenes, and G. Ríos**

IVIA Instituto Valenciano de Investigaciones Agrarias, Moncada, Valencia (Spain)

Perennial trees release bud seasonal dormancy after a genotype-dependent exposure to chilling, activating a cascade of metabolic reactions that results in meristem growth. Ten peach cultivars with low and medium chilling requirements were sampled after the accumulation of 400 hours below 7°C or chilling hours. RNA extracted from flower buds was labelled and hybridized to a microarray slide containing a set of dormancy-related cDNAs obtained by suppression subtractive hybridization. A principal components analysis (PCA) of hybridization signals grouped the cultivars according to their dormancy behaviour. The dormancy-related variables flowering time (FT), percentage of bud break (PBB), and chilling requirement (CR) were measured in the different cultivars and correlated with microarray fluorescence signals to focus our attention on genes related to dormancy. Those cDNA showing a false discovery rate <0.05 and not identified in the previous work were sequenced and their relative expression analyzed by qPCR. The genes coding for the MADS-box transcription factors DAM4, 5 and 6 showed higher expression level in medium-chilling cultivars 'Rose Diamond' and 'Big Top', with a difference of 100 fold with respect to the early cultivars. A relevant number of genes with higher expression in medium chilling cultivars coded for proteins related to ABA and abiotic stress, as AWPM-19-like, AtMYB44-like transcription factor, LEA protein, early responsive to dehydration (ERD)-like, low temperature induced protein, A20/AN1 zinc finger, RSH2-like, dehydrin, drought-induced protein, and ABI5 binding protein (AFP)-like. The putative role of these genes in dormancy release is discussed.

## OPTIMIZATION OF STRAWBERRY POLLEN CULTURE AND ITS MAINTENANCE OF POLLEN GERMINATION CAPACITY

S. Piripireivatlou<sup>1\*</sup>, A. Imani<sup>1</sup>, and S.H. Masomi<sup>2</sup>

<sup>1</sup>Department of Horticulture Science, Abhar branch, Islamic Azad University, Abhar, Iran

<sup>2</sup>Department of Agronomy, Takestan branch, Islamic Azad University, Takestan, Iran

Temperate fruits including small fruits, especially Strawberries are important fruit crops which mostly for fruit set needs the pollination of flowers and followed by pistil fertilization. Therefore, to achieve this act, pollen viability and its germination capability is essential. For that reason, test of pollen viability and understand its quality is important. By the same token, test to optimize the pollen medium of Strawberry and determination best medium for Strawberries with 9 types of culture medium containing different concentrations of boric acid (0,25 and 50 mg/l), sucrose (7, 10.5 and 15%) and agar (2 %) in the In-vitro using random complete design with three replications. The results showed that maximum germination in combination medium B<sub>25</sub>S<sub>15</sub> (25 mg boric acid and 15 % sucrose) with 97% germination was lowest germination medium in combination B0S7.5 (0 mg boric acid and 7.5% sucrose) with 23.33% germination occurred. The viability of pollen Strawberry two months after maintenance at temperatures - 20°C and - 80°C showed that pollen stored at temperatures above maintain viability of pollen germination or no significant difference. Also, the results from pollen germination in selected culture medium showed that maximum pollen germination (98.83%) in laboratory condition was obtained and lowest pollen germination percentage in 10°C with 98.83% occurred. Quantity of pollen germination decreased with increasing time of maintenance of pollen. Results showed that pollens maintaining at 4°C have much germinations rather than 22°C. Therefore, increasing the time of maintaining of pollens need to decreasing the temperatures of maintenance.





**Poster session**

**GENETIC AND GENOMIC STUDIES OF FRUIT  
QUALITY**



## A NOVEL ANTHOCYANIN GENE IN DOMESTIC APPLE

**K.R. Tobutt, C. Bester\*, and J.P. Human**

ARC Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch, 7599, South Africa  
e-mail: besterc@arc.agric.za

An apple progeny from the cross of an ARC apple selection 8A-02-15/16 by the South African cultivar 'Levant' is segregating for a novel acyanic phenotype. Approximately 25% of the seedlings have white flowers with no trace of pink followed by fruit which is green or yellow without any blush; the leaves and shoots are devoid of any red pigmentation. Neither parent shows this phenotype. Thus this acyanic trait can be attributed to a recessive gene, for which the symbol *acy* is proposed. A cultivar in the UK National Fruit Collections, 'Reinette d'Espagne (false)', which has now been grubbed, had a similar phenotype. This trait may be useful in breeding dessert cultivars and ornamental crab-apples.

## GENETIC, BIOCHEMICAL AND MOLECULAR STUDIES OF CAROTENOID ACCUMULATION AND AROMATIC COMPOUND EMISSION IN WHITE- AND YELLOW-FLESHED PEACH GENOTYPES

F. Brandi<sup>1\*</sup>, A. Liverani<sup>1</sup>, D. Giovannini<sup>1</sup>, G. Bianchi<sup>2</sup>, V. Papa<sup>2</sup>, M. Buccheri<sup>2</sup>, F. Mourgues<sup>3</sup>, and C. Rosati<sup>3</sup>

<sup>1</sup>Consiglio per la Ricerca in Agricoltura, Unità di Ricerca per la Frutticoltura-Forlì (CRA-FRF), via la Canapona 1 bis, 47100 Forlì, Italy

<sup>2</sup>CRA-IAA - Unità di Ricerca per i Processi dell'Industria Agroalimentare, Via Venezian 26, 20133 Milano

<sup>3</sup>ENEA, Trisaia Research Center, S.S. 106 km 419+500, 75026 Rotondella (M,T) Italy

Among Rosaceae, Peach (*Prunus persica* L. Batsch) is an appealing model crop. Peaches are appreciated for their visual, nutritional and organoleptic features, partially contributed by carotenoids, sugars, acids and volatile organic compounds (VOCs). In particular, carotenoid accumulation in the mesocarp determines the difference between yellow- and white-fleshed genotypes, the latter being generally characterized by a more intense aroma. Recent research unveiled the presence of carotenoid dioxygenase CCD and NCED enzymes which specifically cleave carotenoid compounds to form volatile norisoprenoids, abscisic acid (ABA) and regulators of plant growth and development. Carotenoid accumulation, VOC emission related to carotenoids cleavage (neral,  $\alpha$ -phellandrene,  $\alpha$ -terpinene, terpinolene, geranyl acetone and  $\gamma$ -terpinene), transcript levels of relevant carotenoids and ethylene production during fruit development were investigated in five genotypes: 'Redhaven' (RH; yellow-fleshed), its white-fleshed mutant 'Redhaven Bianca' (RHB), "Big Top" (BT; yellow-fleshed) and two stony hard (SH) genotypes (IFF 331 and Ghiaccio1). SH fruits do not produce ethylene during ripening; BT starts to produce ethylene at S3 (fruit swelling) stage; RH and RHB at S4 one. The expression patterns of key carotenoid pathway genes during fruit development were determined. In yellow-fleshed RH and BT, early genes of the carotenoid pathway were highly expressed; the expression of the carotenoid dioxygenase genes was, conversely, negligible. Very low transcript levels were found in the two SH genotypes, either for early and for late genes of carotenoid pathway. Finally, in RHB, early genes were highly expressed, although in different fruit developmental stages when compared to the yellow ones; transcript levels of dioxygenase genes in this cultivar were dramatically higher compared to the other genotypes thoroughly fruit development. Total carotenoid concentration in the yellow-fleshed RH and BT progressively increased from stage 2 (S2=pit hardening) up to full ripening (S4) and was much higher than that found in the white-fleshed genotypes. Analysis pointed out strong differences in the accumulation of the distinct VOC compounds in the genotypes studied.

## A PREDICTIVE GENETIC TEST FOR APPLE “FRESH TASTE” PROVIDES STRATEGIES FOR IMPROVED BREEDING AND FRUIT HANDLING

S. Verma<sup>1</sup>, Y. Guan<sup>2</sup>, K.M. Evans<sup>2\*</sup>, J. McFerson<sup>3</sup>, E. van de Weg<sup>4</sup>, J. Luby<sup>5</sup>, S.K. Brown<sup>6</sup>, and C. Peace<sup>1</sup>

<sup>1</sup>Washington State University, Pullman, USA

<sup>2</sup>Washington State University, Wenatchee, USA

<sup>3</sup>Washington Tree Fruit Research Commission (WTFRC), Washington State, USA

<sup>4</sup>Plant Research International, Wageningen, Netherlands

<sup>5</sup>Department of Horticultural Sciences, University of Minnesota, USA

<sup>6</sup>Department of Horticulture, Cornell University, USA

Delicious fresh-tasting apples are highly desired by the market. Developing new varieties that produce such fruit in abundance is the major goal of the Washington apple breeding program. Promising research at WSU may help breeding efficiency and inform industry fruit handling practices. To supply the public with year-round apples, fruit are stored up to 12 months after harvest. Apples genetically predisposed to store well and retain a fresh flavor would be most valuable for the Washington industry (which provides more than half the U.S. supply) and satisfying for consumers. A location in the apple genome (the *Ma* locus, for "malic acid") has been discovered that controls apple taste and texture. We identified variants of the locus that appear to predispose certain varieties to produce fresh-tasting apples. For example, the ultra-crisp and juicy variety Honeycrisp has a unique genotype for the *Ma* locus. Longtime favorite Golden Delicious has the composite genotype of a high-tartness variant and a bland-taste variant. Granny Smith has a double-tartness genotype. Using pedigree-viewing software, we visualized the genotypes of hundreds of apple varieties and integrated observational data on flavor and texture. This analysis revealed predictive genotypes of common varieties and breeding germplasm. One potential outcome of this work is development of fruit flavor/texture genetic categories to optimize postharvest management strategies. Another targeted application is inheritance monitoring of desirable variants during breeding. To facilitate that effort, we seek to confirm a candidate gene for the *Ma* locus arising from examination of the recently released apple whole genome sequence.

## ***In silico* GENOME WIDE SEARCH FOR PUTATIVE GENES OF THE ANTIOXIDANT KEY ENZYMES INVOLVED IN THE PHYSIOLOGICAL ELIMINATION OF ROS**

**P. Irisarri\*, P. Errea, and A. Pina**

Unidad de Fruticultura, Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA), Zaragoza, Spain

Biotic and abiotic stresses such as wounding, heavy metals, illumination, temperature extremes, drought or salt stress cause an increase of reactive oxygen species (ROS) which are related with programmed cell death (PCD). Thus, the accumulation of ROS increase activity of catalase (CAT), ascorbate peroxidase (APX) and superoxide dismutase (SOD) which take part of cell defense mechanism against ROS. In grafted plants, vascular elements are regenerated by complex processes and the rootstock can be successful or incompatible. Whereas the biological formation of incompatible and compatible grafts are similar at the early phase of development, it has been reported that oxidative stress change at the point of union in incompatible herbaceous grafts. In this work, we have performed an *in silico* assessment of genes involved in ROS elimination. APX, SOD and CAT were identified by BLAST searches in Peach, Malus and Arabidopsis genomes. Evolutionary relationships were inferred by phylogenetic analysis of aligned amino acid sequences for each gene family showing that they were highly conserved among the Rosaceae family. Additionally, specific gene expression and enzymatic activity were assayed in different kind of pear and quince tissues used for vegetable grafting studies. The results showed that SOD activity was higher than APX and CAT in callus tissue. Work is currently ongoing to determine gen expression and activity of these antioxidant enzymes in different graft combinations at different developmental stages.

## INTEGRATED OMICS ANALYSIS OF SPONTANEOUS MUTANT OF PEAR SETTING GIANT FRUITS

**K. Isuzugawa<sup>1\*</sup>, T. Takashina<sup>1</sup>, Y. Takahashi<sup>1</sup>, T. Hanada<sup>1</sup>, A. Oikawa<sup>2</sup>, R. Nakabayashi<sup>2</sup>, F. Matsuda<sup>3</sup>, S. Yamaguchi<sup>2</sup>, H. Mori<sup>4</sup>, and K. Shiratake<sup>4</sup>**

<sup>1</sup>Horticultural Experiment Station, Yamagata Integrated Agricultural Research Center, Sagae, Japan

<sup>2</sup>RIKEN Plant Science Center, Yokohama, Japan

<sup>3</sup>Organization of Advanced Science and Technology, Kobe University, Kobe, Japan

<sup>4</sup>Graduate School of Bioagricultural Science, Nagoya University, Nagoya, Japan

Fruit size is an agronomically important trait in pear as well as in other fruits. In spite of its importance, molecular bases of the factors controlling fruit size are poorly understood. To clarify the molecular and cellular mechanisms, we have studied a spontaneous mutant of pear 'La France' setting giant fruits. The observations for two years showed that fruit weight of the mutant was 1.7 - 2.1 times larger than that of wild type. Microscopic analysis revealed that an increase of fruit size was due to cell size but not cell number in the fleshy cortex. Interestingly, we detected 2C, 4C and 8C peaks in receptacle and pedicel at full bloom and 4C peaks in fleshy cortex at harvest by flow cytometry analyses, whereas only 2C peaks in leaves. The unique fruit traits of the mutant, such as high acidity, low starch content and low firmness were observed. The soluble solid contents of the mutant fruits were similar to those of wild type fruits, indicating that sink strength was high. The analyses of this mutant could be potentially informative to clarify the mechanisms of cell enlargement in fruit tissue. In order to know whether the traits are inheritable, we are gaining the progeny crossed with between the mutant and precocious pear line transformed with citrus *FT* gene. Moreover, analyses of transcriptome, proteome, metabolome and hormonome are going on. In this symposium, the outline of our project and the latest results will be presented.

## ANALYSIS OF SUPPRESSION SUBTRACTIVE HYBRIDIZATION FOR DIFFERENTLY EXPRESSED GENES BETWEEN 'REDFIELD' AND 'GREENSLEEVES' APPLE FRUITS

S.E. Han<sup>1,2</sup>, H.E. Lee<sup>2</sup>, S. Heo<sup>2,3</sup>, Y-J. Oh<sup>4</sup>, and D. Kim<sup>4\*</sup>

<sup>1</sup>SolGent Co., Ltd., Daejeon 305-348, Korea

<sup>2</sup>National Institute of Horticultural & Herbal Science, Suwon 440-706, Korea

<sup>3</sup>Healthy Family Support Center, National Plant Quarantine Service, Seoul 121-886, Korea

<sup>4</sup>Department of Horticultural Science, Chungbuk National University, Cheongju 361-763, Korea

Most of the red and blue colors found in higher plants are influenced by anthocyanins and their pathways. Anthocyanin accumulation is regulated by MYB transcription factors. Some varieties of apple indicate anthocyanin accumulation with a red color in the skin and, at times, even in the cortex core, but other varieties without anthocyanin production have green-colored skin indicative of chlorophyll accumulation. To obtain gene expression profiles from the apple cultivars with different flesh and skin colors, 2 apple cultivars, the red-colored 'Redfield' and the green-colored 'Greensleeves', were used. A cDNA suppression subtractive hybridization (SSH) library was established and analyzed. Six genes encoding methallothionein-like protein, chalcone isomerase, dirigent-like protein, brassinosteroid-6-oxidase, an unnamed protein product, and some unknown proteins were selected, and their expression was confirmed in the 'Redfield' cultivar through virtual northern blot analysis. In the 'Greensleeves' cultivar, 5 genes encoding gibberellins-regulated protein, a hypothetical 23.5 KD protein, anthranilate N-hydroxycinnamoyl/benzoyltransferase, and isoflavone reductase were chosen, and their upregulated transcriptional levels were identified. These selected genes were differently expressed in each apple cultivar, suggesting that these genes directly or indirectly regulate anthocyanin accumulation.



## EVALUATION OF NUTRACEUTICAL CONTENT IN FRUIT OF 13 HIGHBUSH BLUEBERRY CULTIVARS

**T. Krupa**

Warsaw University of Life Sciences – SGGW, Faculty of Horticulture and Landscape Architecture, Department of Pomology, Warsaw, Poland

Nutraceuticals are natural bioactive, chemical compounds of health promoting, disease preventing properties. The definition of nutraceuticals was expanded to include vitamins, polyphenols and flavonoids. Flavonoids occur in the form of aglycones, glycosides and methylated derivatives. In plants, aglycone flavonoids (i.e., flavonoids without attached sugar) occur in a variety of structural forms. It is well known that the highbush blueberry fruits are a very rich source of antioxidants. The fruits are very attractive and tasty as well. Berries are picked at a commercial ripening stage. Their total polyphenolic and anthocyanin content, chlorogenic acid, vitamin C content and the effectiveness of binding free radicals using the synthetic radical DPPH were measured. Additionally qualitative and quantitative analyses of individual anthocyanin compounds were performed using the HPLC method. The achieved results confirm the opinion that highbush blueberry fruits are a good source of vitamin C, although considerable differences among particular cultivars were found. The total anthocyanin contents determined in 13 cultivars amounted to from 115 to 253 mg/100 g of fresh matter. Using the HPLC technique for the separation of pigments, 14 colour compounds from the anthocyanin group were detected in the experiment, i.e. delphinidin-3-glucoside, delphinidin-3-galactoside, delphinidin-3-arabinoside, cyanidin-3-glucoside, cyanidin-3-galactoside, cyanidin-3-arabinoside, peonidin-3-glucoside, peonidin-3-galactoside, petunidin-3-glucoside, petunidin-3-galactoside, petunidin-3-arabinoside, malvidin-3-glucoside, malvidin-3-galactoside, and malvidin-3-arabinoside. It was demonstrated in the experiment that not all the analyzed compounds from the anthocyanin group are strongly correlated with the total anthocyanin content. A high correlation was observed in case of delphinidin-3-glucoside, delphinidin-3-galactoside, delphinidin-3-arabinoside, cyanidin-3-galactoside, peonidin-3-glucoside, peonidin-3-galactoside. Antioxidant activity of highbush blueberry fruits correlated with the content of total anthocyanins were noted in case of delphinidin-3-glucoside, delphinidin-3-galactoside, delphinidin-3-arabinoside, petunidin-3-glucoside, and petunidin-3-galactoside.

## USE OF NIR SPECTROSCOPY FOR BREEDING APPLES OF QUALITY AND NUTRITIONAL FEATURES

**A. Pissard, P. Dupont, V. Baeten, G. Sinnaeve, J-M. Romnee, A. Mouteau, and M. Lateur\***

CRA-W, Walloon Agricultural research centre, Life Sciences Department, Breeding and Biodiversity Unit, Walloon Agricultural Research Center, Rue de Liroux, 4, 5030 Gembloux, Belgium

Quality and nutritional parameters represent an increasing concern of many consumers. Vitamin C and some polyphenols are particularly focused as they are strong antioxidants. This concern underlines the need for reliable techniques to evaluate food quality. Here the use of NIR spectroscopy was investigated as a rapid and non-destructive tool which could be applied in breeding program of apples for differentiated quality. The present preliminary results concern the determination of six important quality parameters of apples namely the maturity, firmness, sugar content, acidity, and the vitamin C and total polyphenols content. Thirty-two varieties were collected in 2009 and 2010 to cover the broad variability of the CRA-W's collections. Six fruits per variety collected at the physiological maturity were analyzed. The spectra were acquired in reflectance mode (408 - 2498 nm). After NIR measurements, the maturity, firmness, and acidity were determined according standard protocols. The content of vitamin C was determined by UPLC-MSMS, the total polyphenol content by the Folin-Ciocalteu and the sugar content using a digital refractometer. Calibration models were developed using PLS regression. Best results were obtained for the determination of sugar (SEC = 0.54; Rcal = 0.91), acidity (SEC = 1.60; Rcal = 0.89), and total polyphenols content (SEC = 84.93; Rcal = 0.89). RPD values for these parameters varied between 2.48 and 2.89, which corresponds to models with good precision of prediction. Moreover, it means that almost three groups of values (low, medium and high) may be created when apples are analyzed for breeding purposes. Vitamin C was slightly less performing with SEC of 0.59, Rcal of 0.82 and a RPD of 1.87. These results indicate that NIR is a potential powerful tool for the breeders interested in improving quality and nutritional parameters of fruits. Further works are necessary to improve the precision of prediction models and, in particular, to develop precise calibration models on portable devices directly useful in orchards.

## **FRUIT AND OIL CHARACTERISTICS OF ADVANCED SELECTIONS FROM AN OLIVE BREEDING PROGRAM**

**P.J. León<sup>1</sup>, N. Talhaoui<sup>1</sup>, L. León<sup>1</sup>, L. Velasco<sup>2</sup>, B. Pérez-Vich<sup>2</sup>, and R. De la Rosa<sup>1</sup>**

<sup>1</sup>IFAPA Centro Alameda del Obispo. Avda. Menéndez Pidal, s/n, E-14004 Córdoba, Spain

<sup>2</sup>Instituto de Agricultura Sostenible (CSIC), Apartado 4084, E-14080 Córdoba, Spain  
e-mail: raul.rosa@juntadeandalucia.es

Early bearing, high yield and oil content, mechanical harvesting aptitude, expanded ripening period and oil quality are some of the main objectives of breeding programs aiming at obtaining new olive cultivars for olive oil production. These characters are mainly evaluated in the last steps of the breeding work where a reduced number of genotypes with high number of replications are evaluated. In this work, the agronomic characteristics of 8 advanced selections together with ‘Arbequina’ and ‘Picual’ cultivars as controls were studied. These selections were obtained in the cooperative breeding program carried out by the University of Cordoba and the Institute of Agricultural and Fishery Research and Training (IFAPA). Significant differences between genotypes were observed for all the characters under study. Variation of fruit characters throughout was evaluated once a month from September to December. This allows to compare the ranking of genotypes throughout the ripening period considering different dates or ripening index of samples, and to determine the optimal harvesting date for each genotype based on patterns for oil accumulation, oil quality characters and ease of fruit detachment. Future works will be planned to assess the possibilities of some of the selections evaluated as promising new olive cultivars under different environmental conditions.

## **INVESTIGATION OF VOLATILE CONSTITUENTS IN SOME PROMISING LOCAL PEACH AND NECTARINE GENOTYPES USING HS-SPME TECHNIQUE BY GC-MS**

**M. Seker<sup>1</sup>, E. Gur<sup>2</sup>, and N. Ekinci<sup>2</sup>**

<sup>1</sup>University of Çanakkale Onsekiz Mart, Faculty of Agriculture, Department of Horticulture, Çanakkale, Turkey

<sup>2</sup>University of Çanakkale Onsekiz Mart, Lapseki Vocational College, Lapseki, Çanakkale, Turkey

One of the most important consumer acceptability is fruit aroma in peaches and nectarines. White nectarines are locally grown in the Northwestern region of Turkey. They have small fruits with white-cream color and have high market value because of its unique aroma and attractive appearance. Nine peach and nectarine genotypes including local flat peach, three white nectarines and six standard varieties were investigated for their volatile compositions using SPME (Solid Phase Micro Extraction) technique by GC/MS (Gas Chromatography/Mass Spectrometry). White nectarines, the most aromatic genotypes, were observed to accumulate high levels of esters compared to the other genotypes.

## KERNEL CHEMICAL COMPOSITION OF THE WALNUT GENETIC RESOURCES GROWN IN THE HIGH ATLAS MOUNTAINS OF MOROCCO

O. Kodad<sup>1</sup>, J.P. Wathelet<sup>2</sup>, G. Estopañan<sup>3</sup>, T. Juan<sup>3</sup>, R. Socias i Company<sup>4</sup>, and M. Sindic<sup>5</sup>

<sup>1</sup>Département d'Arboriculture. Ecole Nationale d'Agriculture de Meknès. BP S/40. Morocco

<sup>2</sup>Unité de Chimie Générale. ULg-Gembloux Agro-Bio Tech. Gembloux. Belgium

<sup>3</sup>Unidad de Calidad y Seguridad Alimentaria, CITA de Aragón, Apartado 727, 50080 Zaragoza, Spain

<sup>4</sup>Unidad de Fruticultura, CITA de Aragón, Apartado 727, 50080 Zaragoza, Spain

<sup>5</sup>Unité de Technologie des Industries Agro-Alimentaires. ULg-Gembloux Agro-Bio Tech. Gembloux. Belgium

More than half of the cultivated walnuts (*Juglans regia* L.) in Morocco are seedlings, resulting from the prevailing way of seed propagation known by farmers. Principal Component Analysis (PCA) was used to compare protein, oil content, fatty acid composition and tocopherol isomers among 14 selected clones from the High Atlas Mountains. The results showed a high genetic variability of these components among genotypes, confirming the high genetic diversity of walnut in the Atlas Mountains. Thus, the application of a chemometric approach appears to be useful to evaluate the genetic diversity in walnut. The studied genotypes tended to be characterized by high oleic acid and medium values of  $\gamma$ -tocopherol. Three genotypes with a high oil quality were identified, characterized by high oleic acid content and low values of linolenic acid. No evidence was found to suggest the existence of separate populations within the walnut genotypes from High Atlas Mountains.

## CLONING AND EXPRESSION PATTERNS OF TEN cDNAs ASSOCIATED WITH PEAR FRUIT (*Pyrus pyrifolia*) DURING THE DEVELOPMENT AND RIPENING STAGE

R. Wang, X.N. Zhang, S.L. Yang, J. Zhu, M. Wang, and N.N. Zhao

College of Horticulture and Landscaping, Qingdao Agricultural University, No. 700, Changcheng Road, Chengyang District, 266109, Qingdao, Shandong, China

Phenylalanine ammonia lyase (PAL), 4-coumarate: coenzyme A ligase (4CL), cinnamyl alcohol dehydrogenase (CAD) and peroxidase (POD) are known to be responsible for the lignification in many fruit and vegetables. It is critical to understand the mechanisms underlying the *PAL*, *4CL*, *CAD* and *POD* genes expression patterns during the fruit development and storage, in order to develop strategies that would improve fruit qualities and storage techniques. In the present research, ten cDNAs associated with the lignification pathway, i.e., *PcPAL*, *PpPAL2*, *Pp4CL1*, *Pp4CL2*, *PpCAD1*, *PpCAD2* and *PpPOD1*, *PpPOD2*, *PpPOD3*, *PpPOD4*, were isolated from flesh tissue of Whangkeumbae fruit (*Pyrus pyrifolia* Nakai) by RT-PCR and RACE techniques. The homology alignment analysis showed that there was 84% identity between the amino acid sequences of *PpPAL1* and *PpPAL2*, 66.5% identity between the amino acid sequences of *Pp4CL1* and *Pp4CL2*, 95% identity between the acid sequences of *PpCAD1* and *PpCAD2*, and there was just 44.5% identity among the four POD genes isolated from Whangkeumbae fruit (*Pyrus pyrifolia* Nakai). In terms of relationships with genes which have been demonstrated to have a specific role in lignin biosynthesis, there was particularly close homology between the two PAL genes from pear and *AtPAL2*(P45724) from *Arabidopsis*. The two 4CL genes had homologies ranging from 66 to 70% with *At4CL1* (AAM20598) and *At4CL2* (AAM20546) from *Arabidopsis*, respectively. The two CAD genes had relatively high amino acid homologies with CAD1 genes from tobacco. The four POD genes had only 32 - 43% amino acids identities with known lignin related POD in tomato. The relative expression levels of the ten corresponding genes were examined by semi-quantitative PCR and real-time quantitative PCR. The results showed that, during the development and ripening stage of pear fruit, the expression patterns of *PpPAL2*, *Pp4CL1*, *Pp4CL2* were similar. With high transcript levels at 20 d after anthesis and then decreased with fruit development. After 69 d after anthesis, their expression maintained a relatively low level to the end of fruit ripening. And *PpPAL1*, *PpPOD1*, *PpPOD2* and *PpPOD4* genes showed a similar expression patterns during fruit development and fruit ripening. Their transcript levels with a relative high level at 20 d after anthesis and increased in the following days then peaked at 53 d after anthesis, and then decreased with fruit development. *PpCAD1* and *PpCAD2* genes expression had the same trends. On the other hand, it was found that *PpCAD2* transcript level was obviously higher than *PpCAD1* and *PpPOD4* transcript level was strongly higher than another three POD genes isolated from *Pyrus pyrifolia*. Nakai. Whangkeumbae fruit. More studies on the lignification mechanisms of Whangkeumbae fruit (*Pyrus pyrifolia* Nakai) during fruit development and storage are being conducted for a better understanding of the pear fruit lignification.

## LIGNIN METABOLISM IN RUSSET FRUIT SKIN OF A PEAR MUTANT AND CLONING OF ITS CORRELATED GENE

H. Wei\*, Y. Zhen-feng\*, J. Bing, L. Xiao-feng, L. Xue, and Z. Li-wu

Key Laboratory of Pomology, Anhui Agricultural University, Hefei, 230036 China

The pericarp of 'Dangshansuli' pear was green in young fruit and turned into yellow gradually as fruit mature, while that of 'Xiusu' pear, a mutant of 'Dangshansuli' pear, turned into russet from late July. To study the mechanism of russet skin formation, the lignin contents in fruit skin of both 'Dangshansuli' and 'Xiusu' were measured, the microstructures were also observed, and the four genes, *PAL*, *F3H*, *F5H* and *CCoAOMT*, related with the pathway of lignin biosynthesis were cloned. The results showed that the lignin content in pericarp was 33.03%, when the fruit color of 'Xiusu' turned from green to russet at the late of July, showing significant difference with that of 'Dangshansuli' 23.65%. The lignin contents of 'Dangshansuli' and 'Xiusu' fruit skins at fruit mature were 31.50% and 35.34%, respectively, exhibiting distinct difference. In 'Xiusu' pear, the arrangement of epidermal cells was tighter, the intercellular space was narrower, and the cellular walls were thicker than that in 'Dangshansuli' pear. The inclusions of some epidermal cell in 'Xiusu' were degraded gradually but that in 'Dangshansuli' were normal. Based on the cDNA sequence analysis, the length of *PAL* gene in 'Xiusu' or 'Dangshansuli' pear both are 1188 bp but with 28 base pairs difference and the homology of 97.56%. There are four amino acids difference in 395 ones with the homology of 98.99%. The amino acid sequences of *F3H*, *F5H* and *CCoAOMT* in 'Xiusu' pear had a 100% homology with that of 'Dangshansuli' pear.

## **INFLUENCE OF CALCIUM CONTENT ON LIPOXYGENASE ACTIVITY OF APPLE CULTIVARS 'GALA' AND 'ŠAMPION'**

**M. Wińska-Krysiak**

Laboratory of Basic Research in Horticulture, Warsaw University of Life Sciences – SGGW, Nowoursynowska 159, 02-776 Warsaw

The lipoxygenase (LOX) family of lipidperoxidizing, nonheme iron dioxygenases form products that are precursors for diverse physiological processes in numerous living organisms, bacteria, plants and animals. In plants, LOXs are involved in growth and development including organ development, lipid metabolism, nitrogen storage, plant responses to stress, mechanical wounding and plant-microbe interactions. Because LOX activity depends on the ethylene synthesis, thus leading to hypothesize that this enzyme is involved also in fruit ripening process. It is also believed that occurrence of bitter pit during prolonged storage of apples may be related to the increased activity of LOX. The aim of this work was to investigate the relationship between Ca content, K:Ca ratio and lipoxygenase activity during development and storage of fruits of cultivars known of low ('Gala') and high ('Šampion') susceptibility to bitter pit. Apples and leaves were collected from 4 years old trees of cultivars 'Gala' and 'Šampion' on the M.9 rootstock in the Experimental Orchard of Department of Pomology at Wilanów. Twenty apples of each cultivar were randomly collected: three times during development of apple fruit and twice during common cold as well as CA storage. Significant differences in the mean content of calcium between two cultivars and also between organs were noted. The content of calcium was significantly higher in leaves compared to fruits. In leaves the content of Ca varied from 1.03% d.w. to 2.34% d.w., and in fruits from 246 to 1967 mg·kg<sup>-1</sup> d.w. During the growing season increase of Ca in leaves was noted, while in fruits its content decreased. Mean K:Ca the ratios were 1.22 and 0.66 in leaves and 3.9 and 4.67 in fruits of cultivars 'Gala' and 'Šampion', respectively. LOX activity was higher in leaves than in fruits. Leaf and fruit lipoxygenase activity of 'Gala' was significantly lower than 'Šampion'. During the growing season LOX activity increased, both leaves and fruits, irrespective of the year and cultivar. The progressive increase of LOX activity was also observed during storage of fruit and after shelf life. LOX activity was negatively correlated with the content of calcium.

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**Poster session**

**BIOTECHNOLOGY, MARKER DEVELOPMENT AND  
MOLECULAR BREEDING**



**DEVELOPMENT OF NOVEL MICROSATELLITE MARKERS FROM AN ENRICHED GENOMIC LIBRARY IN *Pistacia Vera* L.**

**S. Kolahi Zenozi<sup>1\*</sup>, M. Mardi<sup>1</sup>, I. Tabatabaei<sup>1</sup>, S. Moftafa Pirseyedi<sup>1</sup>, M. Farsi<sup>1</sup>, M. Zeinalabedini<sup>1</sup>, M. A. Ebrahimi<sup>2</sup>, and S.M.K. Nekoui<sup>1</sup>**

<sup>1</sup>Department of Genomics, Agricultural Biotechnology Research of Iran (ABRII), P.O.Box 31535-1879, Karaj, Iran

<sup>2</sup>Department of Biotechnology, University of Payame Noor, Karaj, Iran  
e-mail: mardi@abrii.ac.ir

Pistachio (*Pistacia vera* L.) is one of the most important horticultural crops in Iran. Here, we report the isolation and characterization of 12 novel microsatellite markers isolated from a repeat-enriched genomic library of *P. vera* L. successful polymorphism of the 12 microsatellite markers was observed in 45 cultivars of *P. vera* L. The number of polymorphic alleles ranged from 2 to 4 (with an average of 0.35). These new markers should allow studies of the population structure and genetic diversity of pistachio to be performed in the future.

## DETECTION AND VALIDATION OF SNPs AND A SFP IN CHLOROPLAST *Prunus* SPECIES

**B. Bielsa<sup>1</sup>, D. Jiwan<sup>2</sup>, A. Dhingra<sup>2</sup>, A. Fernandez-Marti<sup>3</sup>, and M.J. Rubio-Cabetas<sup>1\*</sup>**

<sup>1</sup>Unidad de Fruticultura CITA – Gobierno de Aragon, Avda. de Montañana 930, 50059 Zaragoza, Spain

<sup>2</sup>Department of Horticulture and Landscape Architecture, Washington State University Pullman, Washington 99164, US

<sup>3</sup>Fundación Parque Científico Tecnológico Aula Dei, Av. de Montañana, 930, 50059 Zaragoza, Spain  
e-mail: mjrubioc@aragon.es

*Prunus* spp. is a very wide genus including all the stone fruits. Peach, almond, plum, apricot and cherry, are mainly grown in the Mediterranean regions. The amplification, sequencing and annotation of plastomes (ASAP) technique has been used to identify polymorphism between different genotypes of rootstocks because it is a simple and an inexpensive method. The genotypes studied were myrobalan plums (*P. cerasifera* Ehrh), and almond × peach hybrid (*P. amygdalus* × *P. persica*), used as control rootstock for many abiotic response like chlorosis, drought and waterlogging tolerance. The amplification and sequencing of the IR region of 25960pb plastid genome using the ASAP technique, revealed single-nucleotide polymorphisms (SNPs) in the 3' end of the IR B region. The SNPs were common between the almond and peach genotypes but different from the myrobalan progeny. In addition, a SFP-indel of 18 nucleotides was found in the 5' end IR B region. This indel was present in the hybrid 'GF-677' but absent in the myrobalan genotype 'Myrobalan 29C'. Primers were designed to identify and amplify such as indel generating a 182 bp fragment in the almond x peach hybrid genotypes and a 165 pb fragment in the plum genotypes. Several other almond x peach hybrid rootstocks ('Garnem', 'Felinem') and plum genotypes ('P.2175' and 'P.2980') have been also tested in order to validate the indel polymorphism. This single-feature polymorphism (SFPs) has been further used to identify several 3-way hybrids created in a breeding program for the characterization of new interspecific plant material. It is noteworthy that the chloroplast genome region where these polymorphisms have been identified is distinct from the regions where most of the chloroplast microsatellites (SSRs) have been identified previously by several other groups. In this paper we are presenting the detection and validation of such a indel in several interspecific hybrids, between plum and almond × peach hybrids.

## SNP DEVELOPMENT ON A RESEQUENCED PEACH GENOTYPE FOR MAPPING AND QTL ANALYSIS

S. Tartarini<sup>1\*</sup>, L. Dondini<sup>1</sup>, R. Paris<sup>1</sup>, G. Pagliarani<sup>1</sup>, S. Venturi<sup>1</sup>, S. Sansavini<sup>1</sup>, F. Gennari<sup>1</sup>, E. Marasco<sup>2</sup>, V. Mantovani<sup>2</sup>, R. Pirona<sup>3</sup>, I.P. Cruz<sup>3</sup>, L. Rossini<sup>3</sup>, S. Foschi<sup>4</sup>, S. Scalabrin<sup>5</sup>, F. Cattonaro<sup>5</sup>, D. Bassi<sup>6</sup>, and I. Verde<sup>7</sup>

<sup>1</sup>Dipartimento Colture Arboree Università di Bologna

<sup>2</sup>Centro Ricerca Biomedica Applicata CRBA Policlinico S.Orsola-Malpighi, Università di Bologna

<sup>3</sup>Parco Tecnologico Padano, Lodi

<sup>4</sup>CRPV

<sup>5</sup>Istituto di Genomica Applicata, Udine

<sup>6</sup>Dipartimento di Produzione Vegetale Università di Milano

<sup>7</sup>Consiglio per la Ricerca e la Sperimentazione in Agricoltura, Roma

The recent delivery of the whole peach genome sequence by the International Peach Genome Initiative (IPGI), together with the Italian project "DRUPOMICS" has opened new frontiers in peach genomics. The re-sequencing of single peach genotypes with high throughput methods become now feasible because the whole genome sequence can drive the efficient alignment of the short Illumina readings. The re-sequencing of the parent(s) of a mapping progeny and its alignment to the reference sequence will facilitate the development of polymorphic markers in intra-specific crosses where the degree of polymorphism is known to be a limiting factor. In the framework of the Drupomics project, a peach F1 accession derived from the cross 'Contender' x 'Ambra', named F1CxA, was re-sequenced by Illumina technology with about a 20X coverage. Several thousands of SNPs were identified by aligning the Illumina readings with the peach reference sequence. About two hundreds of genome wide well-distributed SNPs have been used to genotype a peach progeny of 306 seedlings derived from the self-pollination of the F1CxA by a Sequenom platform. A perfectly anchored molecular map spanning the whole peach genome was constructed. Linkage group denominations were also confirmed by the addition of some SSRs. Markers order substantially confirms their expected positions on the first draft of the genome sequence with a few exceptions that will be useful for a second release of the peach sequence. The fruits of each seedling of the CxA progeny were also phenotyped for some quality traits and these data have been used for QTL analysis.

## DEVELOPMENT OF SNP MARKERS FOR MARKER-ASSISTED SELECTION IN PEAR

S. Terakami\*, Ch. Nishitani, and T. Yamamoto

National Institute of Fruit Tree Science, National Agriculture and Food Research Organization (NARO), 2-1 Fujimoto, Tsukuba, Ibaraki 305-8605, Japan

SNPs are considered as desirable DNA marker for marker-assisted selection because of their advantages of high abundance, co-dominant inheritance, locus specificity, and automated high-throughput analysis. The average number of SNPs in introns has been reported to be several times higher than that in exons in many plants and human. In general, introns can be identified by aligning EST sequences with the corresponding genome sequences, however, the genome sequences were limited in public databases in pear. Potential intron polymorphism (PIP) markers, which were designed from EST sequences (exon regions), could amplify potential flanking intron regions without genome sequence information. In the present study, we applied PIP markers designed from apple ESTs on pear, for identification of intron regions and detection of SNPs in pear. A total of 288 PIP markers (MdomPIP1–MdomPIP288) were evaluated for the Japanese pear (*Pyrus pyrifolia* Nakai) ‘Housui’ and the European pear (*P. communis* L.) ‘Bartlett’. Out of 288 PIP markers tested, 252 markers amplified fragments in both cultivars. The fragment size was larger than the size predicted from apple EST sequences, suggesting the existence of intron sequences in amplified fragments. After amplified fragments were sequenced, candidate SNPs in intron regions were identified in genetic linkage maps of ‘Housui’ and ‘Bartlett’. In total, nucleotide sequences of the 68 genomic regions were identified for both ‘Housui’ and ‘Bartlett’. The obtained genome sequences from pear were aligned to the EST sequence of apple, revealing that all genome sequences included donor and acceptor sites, and contained deduced exons and flanking intron regions in pear. The PIP markers designed from apple ESTs were successfully applicable in pear and were useful for detecting SNPs on intron region and developing SNP markers in pear. PIP markers will help us to conduct marker-assisted selection for disease resistances and other important phenotypic traits.

**HEAT-INDUCED PRECOCIOUS FLOWERING OF APPLE PLANTS CONTAINING THE *flowering locus t* GENE FROM POPLAR (*Populus trichocarpa*)**

**S. Wenzel<sup>1</sup>, H. Flachowsky<sup>1\*</sup>, S.H. Strauss<sup>2</sup>, and M.-V. Hanke<sup>1</sup>**

<sup>1</sup>Julius Kühn-Institut (JKI), Bundesforschungsinstitut für Kulturpflanzen, Institut für Züchtungsforschung an gartenbaulichen Kulturen und Obst, 01326 Dresden, Germany

<sup>2</sup>Department of Forest Ecosystems and Society, Oregon State University, Corvallis, OR 97331-5752, USA

Breeding of apple remains a slow process due to protracted generation cycles. Shortening the juvenile phase is a great challenge to achieve the introgression of traits from wild species into pre-breeding material in a reasonable time frame. Within the last ten years the evidence has been adduced that the juvenile phase of fruit trees can be effectively shortened by using gene transfer technologies. In 2007 we succeeded in breaking the juvenile phase in apple by over-expressing the *BpMADS4* gene, a *FRUITFUL*-like gene of silver birch (*Betula pendula* Roth.) in the apple cultivar ‘Pinova’ and subsequently a crossbred-breeding program based on early flowering plants and marker-assisted selection was initiated. Expression of the putative ‘florigen-like’ gene *FLOWERING LOCUS T* (*FT*) has been shown to accelerate the onset of flowering in a number of plant species, including the tree species poplar. Recently we introduced the *FT* genes from poplar (*PtFT1*, *PtFT2*) into the apple cultivar ‘Pinova’, all driven by the heat-inducible promoter *Gmhsp 17.5-E* (*HSP*) from soybean. We investigated the flowering response of the transgenic apple plants obtained using different induction conditions.

## DNA MARKERS DEVELOPED FROM GENOME SEQUENCING ANALYSIS IN JAPANESE PEAR (*Pyrus pyrifolia*)

T. Yamamoto<sup>1\*</sup>, S. Terakami<sup>1</sup>, S. Moriya<sup>2</sup>, K. Kurita<sup>3</sup>, H. Kanamori<sup>3</sup>, Y. Katayose<sup>4</sup>, T. Saito<sup>1</sup>, and C. Nishitani<sup>1</sup>

<sup>1</sup>National Institute of Fruit Tree Science, 2-1 Fujimoto, Tsukuba, Ibaraki 305-8605, Japan

<sup>2</sup>National Institute of Fruit Tree Science, Apple Research Division, Shimokuriyagawa, Morioka, Iwate 020-0123, Japan

<sup>3</sup>STAFF Institute, 446-1 Ippaizuka, Kamiyokoba, Tsukuba, Ibaraki 305-0854, Japan

<sup>4</sup>National Institute of Agrobiological Sciences, 2-1-2 Kannondai, Tsukuba, Ibaraki 305-8602, Japan

A random shotgun sequencing was performed to obtain genome sequences in Japanese pear. Genomic DNAs of the Japanese pear cultivar 'Housui' were analyzed by Roche/454 Genome Sequencer (GS)-FLX Titanium platform. A total of 2.65 million single read sequences were obtained with an average read length of 374 base. More than 990 Mb of nucleotide sequences were recovered, which correspond twice of Japanese pear genome. About 240 SSR markers with tetra- and penta- nucleotide motifs were developed based on more than 50,000 simple sequence repeats found. Out of 240 markers, 138 were newly mapped in at least one of three genetic linkage maps of European pear 'Bartlett' and 'La France', and the Japanese 'Housui'. In addition, 16 retrotransposon-based markers, 86 apple EST-SSRs and 16 SSRs from apple genome sequences were also applied and mapped in pear. An established genetic linkage map of 'Bartlett' consisted of 485 loci from SSR and SNP markers, covering 17 linkage groups in a total length of 965 cM. Other genetic linkage maps of 'La France' and 'Housui' contained 370 loci on 17 linkage groups in a total length of 1,160 cM, and 415 loci on 20 linkage groups in a total length of 1,177 cM, respectively. Genome sequencing analysis combined with 454 EST sequences revealed 5,100 candidate SNPs generated from ca. 16,000 contigs, which will be applicable to high-throughput genotyping. The obtained information will help us to develop genome-wide markers and to perform marker-assisted selection in pear breeding programs. This work is partially supported by a grant from the Ministry of Agriculture, Forestry and Fisheries of Japan (Genomics for Agricultural Innovation, SGE-1003).



## PROFILING TRANSCRIPTS OF DEFENSE RELATED RESPONSE GENES FROM *Vitis coignetiae* NATIVE TO KOREA

H.K. Yun\*, S.Y. Ahn, Y.J. Choi<sup>1</sup>, S.H. Kim<sup>1</sup>, and Y. Y. Hur<sup>1</sup>

Department of Horticultural Science, Yeungnam University, Gyeongsan 712-749, Republic of Korea

<sup>1</sup>National Institute of Horticultural and Herbal Science, RDA, Suwon 440-706, Republic of Korea

*Vitis coignetiae*, a kind of Korean domestic wild grapes, has been known to be resistant to diseases. For screening useful resources for disease resistant grape breeding, cDNA library was constructed from matured leaves and fruits of wild grapes. A total of 2,982 unigenes containing 676 contigs and 2,306 singletons were obtained by sequencing 5,760 expressed sequence tag (EST) clones derived from cDNA library of wild grape, *V. coignetiae*. In gene ontology analysis, 2,241 genes with molecular functions were annotated into 1,442 biological process, and 836 cellular component genes. Defense-related genes including putative proline-rich cell wall protein, thaumatin-like protein, class IV chitinase, and pathogenesis-related protein 10, and photosynthesis-related genes such as ribulose-bisphosphate carboxylase oxygenase small subunit, and water stress-related genes such as abscisic stress ripening protein and aquaporin were the most abundant in cDNAs from grapes and leaves of *V. coignetiae*. These data could provide useful information in genetic analysis of *V. coignetiae* and in the program for breeding disease resistant grape cultivars.

## DEEP RE-SEQUENCING OF APRICOT GENOME TOWARD GENOME-WIDE ASSOCIATION ANALYSIS IN STONE FRUIT SPECIES

T. Zhebentyayeva<sup>1</sup>, Ch. Dardick<sup>2</sup>, J.M. Soriano<sup>3</sup>, E. Zuriaga<sup>3\*</sup>, M.L. Badenes<sup>3</sup>, and A.G. Abbott<sup>1</sup>

<sup>1</sup>Department of Genetics and Biochemistry, Clemson University, 100 Jordan Hall, Clemson, SC 29634, USA

<sup>2</sup>USDA, ARS Appalachian Fruit Research Station, 2217 Wiltshire Road, Kearneysville, WV 25430, USA

<sup>3</sup>Instituto Valenciano de Investigaciones Agrarias, Apartado Oficial 46113 Moncada, Valencia, Spain  
e-mail:tzhebe@clemson.edu, aalbert@clemson.edu

Due to a moderate level of polymorphism, the availability of wild germplasm and a well-described history of dissemination from its primary centers of origin, apricot provides one of the best model systems among the *Prunus* species for marker-trait association analysis. Here, we tested the potential of the Illumina platform (paired-ends reads, barcoded libraries) coupled with CLC software (<http://www.clcbio.com>) for reconstruction of apricot genomes using 2 different strategies- 'de novo' assembly and mapping apricot reads against the *P. persica* v1.0 assembly (<http://phytozome.org>). For this pilot experiment, we selected eight apricot genotypes representing 6 domesticated cultivars (Stark Early Orange, Harlayne, Goldrich, Krasnoshchekii, Real d'Imola, Shalakh) and 2 non-domesticated species *P. mume* and *P. sibirica* var  *davidiana*. Cultivar selection relied on global germplasm analysis by Zhebentyayeva et al. (2011) to address the domestication-related questions - the fruit quality, seed taste, tree architecture, disease resistance and eco-geographical adaptation to fluctuating environments. In this presentation, we report results on genome-wide mapping sequences from 6 domesticated apricots (pooled reads) that allowed aligning 83% out of 376 mln reads against the peach genome. The low proportion of gene-derived sequences among unmapped reads (less than 10%) was in agreement with the known genetic similarity of both genomes from genetic mapping studies. The total genome coverage of  $\sim 80 \times$  (or  $\sim 13 \times$  per genotype) captured the entire peach exom with expected allele representation and was sufficient for genome-wide SNP/indel detection across the entire apricot genome. Fixed sets of markers for regions of breeding interest were generated for haplotype verification in germplasm and breeding material.

**Poster session**

**GENETIC AND GENOMIC STUDIES OF BIOTIC STRESS**



## IS TOLERANCE TO *Penicillium expansum* ASSOCIATED WITH RIPENING DATE AND FRUIT FIRMNESS IN APPLE?

M. Ahmadi-Afzadi<sup>1\*</sup>, I. Tahir<sup>2</sup>, J. Sehic<sup>1</sup>, and H. Nybom<sup>1</sup>

<sup>1</sup>Department of Plant Breeding and Biotechnology, Swedish University of Agricultural Sciences, Balsgård, Fjälkestadvägen 459, SE-291 94 Kristianstad, Sweden

<sup>2</sup>Department of Plant Breeding and Biotechnology, Swedish University of Agricultural Sciences, P.O. Box 103, SE-23053, Alnarp, Sweden

Organic apple production suffers severe economic losses during storage because of postharvest diseases, e.g. blue mould caused by *Penicillium expansum*. Not much data is available from comparative trials on susceptibility for fruit storage diseases. Apple cultivars, however, differ strongly in two traits that may have importance for their susceptibility to blue mould; fruit firmness and ripening time. Therefore, a large set of apples including both early and late ripening cultivars were evaluated in this study. First, optimal harvesting time was determined by an iodine starch test. Fruits of each cultivar were then harvested at a suitable date and inoculated on both sides of the fruit with *Penicillium expansum*. Then, all fruits were stored in a regular cold storage facility (approx. 4 °C) for either 6 weeks (early ripening cultivars) or 12 weeks (late ripening cultivars). Decay severity was evaluated on both sides of infected apples at the end of the storage period. In addition, firmness of the fruits was measured with a penetrometer for each cultivar, both at harvest and after cold storage. The obtained data indicated a strong negative association between harvesting date and amount of blue mould infection (Pearson correlation = -0.631 and -0.509 for early and late ripening cultivars, respectively, P-value < 0.001). Significant correlations were also found between the amount of blue mould infection and initial firmness as well as fruit softening in early ripening cultivars. Late-ripening cultivars with high levels of firmness and little softening were, as expected, the least affected by blue mould. From a breeding point of view, the most interesting cultivars are, however, those that had relatively small symptoms in spite of being early-ripening and/or only medium firm, like Prima, Reglindis, Redfree and Lobo. These cultivars may have other traits that protect them from the fungal infection.

## APPLICATION OF P GENE DONORS IN BREEDING OF BLACK CURRANT RESISTANT TO GALL-MITE

V. Bendokas\*, I. Mazeikiene, D. Baniulis, V. Stanys, and T. Siksnianas

Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry,  
Babtai LT-54333, Kaunas reg., Lithuania  
e-mail: v.bendokas@lsdi.lt

Black currants constitute the most important small fruit crop for horticultural production and present a popular choice for home orchards in Lithuania. Twenty-eight black currant cultivars were released and a large number of perspective progeny was developed during over fifty years of black currant breeding program at the Institute of Horticulture, LRCAF. Resistance to pests and diseases is one of the major goals for black currant breeding, and resistance to gall-mite and fungal diseases were the most important traits for development of new genetic lines and cultivars. An investigation of inheritance of disease resistance in interspecific hybrids of genus *Ribes* revealed species useful as donors for gall-mite resistance genes. Although crosses of *R. nigrum* ssp. *sibiricum*, *R. usuriensis* and *R. Janczewski* resulted in F<sub>1</sub> hybrids resistant to gall-mite, however no hybrids with agronomical value were identified. Further emphasis was put on the development of complex genotype including traits from the European and Siberian black currant genomes and selection for field resistance through several generations. Crossing of *R. nigrum* ssp. *europaeum*, *R. dikusha* and *R. nigrum* ssp. *sibiricum* descendants in two generations resulted in development of several resistant cultivars, with 'Dainiai' being the cultivar of high fruit quality and complete resistance to gall-mite. Approximately 10 percent of hybrids of high breeding value were obtained from crosses including 'Dainiai'.

## INCREASING IMPORTANCE OF PESTS ON Highbush BLUEBERRY PLANTATIONS IN POLAND

A. Chorąży

Department of Applied Entomology, Faculty of Horticulture and Landscape Architecture, Warsaw University of Life Sciences – SGGW, Nowoursynowska 159, Warsaw, Poland

First high bush blueberry (*Vaccinium corymbosum* L.) plantations in Europe were established in 1923 and in 1975 in Poland. During first years of blueberry production, growers did not notice disease and pest on their plantations in Poland. Presently blueberry growers are often facing pest problems, especially on older plantations. The growers also noted that numerous appearance of these pests contributed to significant reduction in the crop quality and quantity. The first report on the pests on blueberry plantations in Poland causing foliage damages mentioned larval stages of some *Lepidoptera* and aphids: *Aphis fabae* Scopoli. According to the growers aphids are presently occurring on the majority of plantations. Also leaf rollers (Tortricidae) and tip midges (*Dasineura*) were often noticed by growers. Other pests such as weevils (*Otiornychus*), fruit worms (*Grapholitha packardi* Zeller), chafers and scales are also noted. On some plantations acarids, butterfly caterpillars (*Lepidoptera*), blossom weevils (*Anthonomus*) and European mole crickets (*Gryllotalpa gryllotalpa* Linnaeus) have appeared. In countries, where high bush blueberry is grown for longer period, pests as well as diseases, chemical control by synthetic pesticides is recommended. Presently the pro-ecological pest management requires: (a) proper identification of pest species and damage symptoms; (b) regular field scouting; (c) using various monitoring techniques and (d) establishing and applying damage thresholds to decide on a proper choice of treatments. The research project started in our Department in 2010 and will focus on the missing information to develop the IPM for blueberry crop in Poland based on: (a) detailed knowledge of entomo- and acaro- fauna occurred on blueberry plants; (b) enhancement of plant resistance/tolerance by bio-stimulators and (c) selection of pest resistant or tolerant plants from the blueberry available germplasm.

## SCREENING OF APPLE CULTIVARS FOR RESISTANCE TO EUROPEAN CANCKER, *Nectria galligena*

L. Garkava-Gustavsson<sup>1,2\*</sup>, A. Zborowska<sup>2</sup>, J. Sehic<sup>1</sup>, M. Rur<sup>3</sup>, H. Nybom<sup>1</sup>, E. van de Weg<sup>4</sup>, and A. Holefors<sup>2</sup>

<sup>1</sup>Swedish University of Agricultural Sciences, Department of Plant Breeding and Biotechnology, Balsgård, Fjälkestadsvägen 459, 29194 Kristianstad, Sweden

<sup>2</sup>Swedish University of Agricultural Sciences, Department of Plant Breeding and Biotechnology, Box 101, 230 53 Alnarp, Sweden

<sup>3</sup>Swedish University of Agricultural Sciences, Department of Plant Protection Biology, Box 102, 230 53 Alnarp, Sweden

<sup>4</sup>Plant Research International, P.O. Box 16, 6700AA, Wageningen, Netherlands

European canker, caused by the fungus *Nectria galligena*, is a severe problem in apple production both in Sweden as well as in many other North-European countries. Even when applying fungicides and good horticultural practices, canker damage occurs almost yearly in nurseries and orchards. Some years, devastating outbreaks can destroy a large numbers of trees. To date, complete resistance to *N. galligena* is not known to occur in apple. Therefore it is important to detect quantitative differences in resistance among cultivars. In the present project 50 apple cultivars were screened for resistance to *N. galligena*. One-year-old shoots from mature trees were inoculated in the greenhouse with a standardised volume of macroconidia suspension using different inoculation methods. Furthermore, 2-year-old trees of 5 cultivars were inoculated in the field. The length of the occurring cankers was measured at regular time intervals throughout a period of two-three months. Cultivars showed differences in colonization rate. In cultivars known to have a high degree of resistance, i.e. Golden Delicious and Red Gravensteiner, lesions progressed much slower compared with notoriously susceptible cultivars like Cox Orange and Elise. These results thus confirm the applicability of the performed resistance test. Results on the entire set of 50 cultivars will be presented and discussed. The just described resistance test is demanding in labour and time (duration). We are therefore exploring alternative approaches including qPCR based assessment of fungal biomass at early stages of infection. Some preliminary results on this will be presented.



## DIVERSITY OF RESISTANCE TO *Monilinia laxa* AND SPRING FROST IN SOUR CHERRY GERMPLASM

**D. Gelvonauskiene\*, B. Frercks, I. Stepulaitiene, and V. Stanys**

Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry,  
Babtai LT-54333, Kaunas reg., Lithuania  
e-mail: d.gelvonauskiene@lsdi.lt

Sour cherry (*Cerasus vulgaris* Mill., syn. *Prunus cerasus*) is one of the most widely grown stone-fruit in Lithuania. Blossom blight (*Monilinia laxa* (Aderh. et Ruhl.) Honey) has spread over the last decade through the country. Blossom blight and spring frost are the major factors influencing yield of sour cherry. Fifty five sour cherry accessions different in origin and resistance to blossom blight were investigated in the collection of Institute of Horticulture, LRCAF during 2001 - 2010. Investigation was carried out in order to identify donors of resistance to blossom blight and to study relation between flower damage during spring frosts and infection of blossom blight. Investigation was performed in the natural background of pathogen. The sensitivity of cultivars to blossom blight was evaluated according to a number of injured one year shoot on a fruit tree: 0– healthy shoots; 5- injured more than 75% shoots. All the studied accessions were grouped according intensity of maximum injury: resistant (injury up to 1 point), moderately resistant (injury up to 2 points), moderately susceptible (injury up to 3 points), susceptible (injury up to 4 points) and very susceptible (injury up to 5 points). Resistance to spring frosts of some cultivars from each group was evaluated in controlled environment. It was established, that blossom blight injured all the accessions of sour cherry, although cultivar resistance to blossom blight, stability of the resistance and dynamics of infection was different. 14.5 percent of studied cultivars were resistant to blossom blight, 9.1 percent moderately resistant, 29.1 percent moderately susceptible, 12.7 percent susceptible and 32.7 percent of cultivars were very susceptible. Flowers and fruit ovaries of ‘Note’, ‘Shirpotreb chiornaya’, ‘Biruliovskaya’ and ‘Maliga empole’ were the most resistant to spring frosts. It was shown that resistance to spring frost and blossom blight correlates.

## **INHERITANCE OF PARTIAL RESISTANCE TO APPLE SCAB IN 10 APPLE PROGENIES**

**C.R. Hampson**

Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, Summerland, B.C., Canada V0H 1Z0

Ten apple progenies were created using a  $2 \times 5$  factorial mating design with the goal of studying the inheritance of partial resistance to apple scab (*Venturia inaequalis*). Two scab-susceptible breeding selections with good fruit quality (both of 'Splendour'  $\times$  'Gala' parentage) were used as seed parents and the following cultivars were used as pollen parents: 'Gewurzluiken', 'Kardinal Bea', 'La Paix', 'Lubsk Queen' and 'Margil'. One hundred plants per family were planted in a randomized complete block design in a plot with a moist maritime climate at Agassiz, B.C., Canada. Commercial cultivars, parent clones and scab race indicators were included in the planting. No fungicides were applied. Scab severity was low over the whole planting in the first year of data collection (2009), but in 2010, the weather was highly conducive to scab infection. Leaf scab symptoms were severe on susceptible commercial cultivars and a high proportion of the progeny plants. Nearly half of the trees in both families with 'Lubsk Queen' as a pollen parent were either symptomless or showed low infection severity. None of the pollen parent clones in the plot showed severe levels of leaf scab, but both female parent clones did. The results are discussed in the context of breeding for durable scab resistance.

## COMPARATIVE STUDIES ON THE BEHAVIOR OF HYBRID APPLE PROGENIES RESISTANTS TO VENTURIA INAEQUALIS UNDER FIELD AND "in vitro" CONDITIONS

L. Ion<sup>1</sup>, D. Hoza<sup>1</sup>, Gh. Petre<sup>2</sup>, V. Petre<sup>2</sup>, C. Moale<sup>3</sup>, V. Lupu<sup>1</sup>, and T. Neagu<sup>1</sup>

<sup>1</sup>University of Agronomy Sciences and Veterinary Medicine Bucharest, Marasti Street, no. 59

<sup>2</sup>Fruit Research & Development Station Voinesti, Romania

<sup>3</sup>Fruit Research & Development Station Constanta, Romania

e-mail: ligiadion@yahoo.com

Fungal diseases include scab (*Venturia inaequalis*), mildew (*Podosphaera leucotricha*) and european canker (*Necria galligena*) are controlled chemically. The priority is to reduce pesticide levels for environmentally safe fruit production. In the short term, new varieties resistant to scab, tolerant to mildew and fire blight should be released at SCDP Voinesti in collaboration with. University of Agronomy Sciences and Veterinary Medicine Bucharest. The breeder has to look for next varieties with both, an easy training system and resistance to the main diseases and pests. The juvenile phase of seedlings is long (5 - 7 years); the large tree size requires much space for field trials. Because of all these reasons, the genetic improvement in this crop is very slow and limited; it takes about 15 years to realize a new variety. Using the techniques *in vitro* multiplication of hybrid progenies of interest in further studies on genetic resistance to scab, with a low susceptibility to mildew and fire blight, quality of the fruit is also a top priority. It is clear selection criteria should also include high productivity, regular shape and uniform bearing of the year.

## EVALUATION OF RESISTANCE TO SHARKA (*Plum pox*) IN SOME ROMANIAN APRICOT PROGENIES

**L. Ion**

University of Agronomy Sciences and Veterinary Medicine Bucharest, Marasti Street, no. 5959

e-mail: ligiadion@yahoo.com

Sharka (plum pox) is considered one of the most devastating diseases of stone fruits in terms of agronomic impact and economic importance (Dunez&Sutic, 1988; Nemeth, 1994). The disease is very detrimental in apricot, peach and plum trees because it produces reduced quality and premature dropping of fruits. The response of the apricot hybrids genotypes was evaluated along of vegetative period of 2010 by visual monitoring of symptoms development and by serological and molecular methods. For testing, leaves samples were collected from the base half of the plants and from the top half of the plants developed from genotype buds. In the case of shoots derived from inoculum buds, leaves were also collected to confirm the PPV presence. After two cycles of study, all the replicates of ‘Mari de Cenad’ showed sharka symptoms while the replicates of ‘Stark Early Orange’ and “NJA 2” did not show any symptoms and were not ELISA-positive. The resistant progenitors (‘Stark Early Orange’ and “NJA2”) was able to transmit its PPV resistance to the descendants, in agreement with previous results observed by other authors. The genetic control hypotheses for PPV resistance in apricot referenced by different authors considered the resistance allele as dominant. Romanian apricot F1 and F2 progenies evaluated were initially classified into two groups: susceptible to PPV and resistant to PPV. After that the most important genotypes were grafted onto infected GF 305 in greenhouse conditions. The next step at this work is the introduction and development the marker – assisted selection (MAS) in PPV resistances F1 and F2 progenies in apricot Romanian genotypes. In all hybrid genotypes and also their parents were isolated samples of DNA to be used to implement the molecular markers.

## IMPROVEMENT OF ROMANIAN OLD VARIETIES OF APPLE AS GENITORS IN OBTAINING HYBRIDS PROGENIES WITH GENETIC RESISTANCE TO *Venturia inaequalis*

**L. Ion**

University of Agronomy Sciences and Veterinary Medicine Bucharest, Marasti Street, no. 59

e-mail: ligiadion@yahoo.com

Scab is a harmful disease in the apple orchard. It is caused by a biotrophic ascomycetes fungus *Venturia inaequalis* (Cke) Wint. In perennial fruit tree species, aspects like the control of pests and disease or fruit maturation require conventional but expensive and time-consuming breeding programmes in order to incorporate the desired characteristics without affecting long-time selected and still valuable traits. The utilization of any potential natural sources of *Venturia inaequalis* resistance remain important for the development of new varieties. For efficient breeding programs to obtain cultivars resistant to scab, it is important to know the genetic control of this resistance. Although there is controversy about the genetic control of the resistance to scab in apples, all authors consider that resistance could be transmitted from resistant progenitors to offspring. However, the descendants from crosses between susceptible and resistant cultivars segregated in a complex way. A new breeding program in apple trees was started in spring 2010, with the interest in introducing resistance to pest and pathogenesis using like genitors, the most adapted varieties (old varieties) at the Romanian conditions which can offer a new vision to the natural genetic resistance, with a positive impact on the environment and the vulnerability of genetic stability at these varieties.

## **IMPROVEMENT OF ROMANIAN ROOTSTOCKS GENOTYPES TO RESTRICT THE PPV INFECTION IN SOME APRICOT PROGENIES**

**L. Ion<sup>1\*</sup>, D. Hoza<sup>1</sup>, C. Moale<sup>2</sup>, I. Zagrai<sup>3</sup>, L. Zagrai, V. Lupu<sup>1</sup>, and S. Preda<sup>4</sup>**

<sup>1</sup>University of Agronomy Sciences and Veterinary Medicine Bucharest, Marasti Street, no. 59

<sup>2</sup>Fruit Research & Development Station Constanta, Romania

<sup>3</sup>Fruit Research & Development Station Bistrita, Romania

<sup>4</sup>Fruit Research & Development Station Valcea, Romania

e-mail: ligiadion@yahoo.com

Sharka disease, caused by the plum pox virus (PPV), is one of the major limiting factors for stone fruit crops in Europe and America. In particular, apricot is severely affected suffering significant fruit losses. The PPV spread throughout the country, seriously affecting apricot cultivation, as all native cultivars were susceptible to PPV. Using local rootstocks that are well adapted to Romanian conditions and which have proved to be resistant to PPV can be a good premise for limiting infection with this quarantine virus. The peach 'GF 305' rootstocks were used as indicators for susceptibility to PPV in comparison with the Mirobolan BN 4 Kr considered to be resistant to sharka. The subsequent grafting protocol was optimized, and a Romanian PPV-D isolate was identified and used as inoculum source. The two rootstocks were grafted with apricot individuals originating from crossing between a PPV resistant genitor (e.g. 'SEO', 'NJA2') and Romanian preferred varieties. The booth rootstocks were inoculated with PPV-D strain by chip-budding. Grafted plants, (GF305 rootstocks and Mirobolan BN 4 Kr) and also apricots hybrids were monitored by visual inspection and ELISA, completed by IC/RT-PCR for the PPV symptoms.

## APPLE CULTIVARS AND HYBRIDS RESISTANT TO NECTRIA CANKER AS INITIAL MATERIAL FOR BREEDING

Z.A Kazlouskaya\* and Y.G. Marchuk

The Republican Research and Production Unitary Enterprise «Institute for Fruit Growing», Samokhvalovichy, Minsk reg., 223013 Belarus  
e-mail: zoya-kozlovskaya@tut.by

One of the main aims in apple breeding in Belarus is resistance to diseases. Increase of damage caused by nectria canker (*Nectria galligena* Bres.) in apple orchards of Belarus was an incentive for starting a wide evaluation of genotypes for resistance to this pathogen. The resistance to nectria canker was investigated within the collection of apple species, hybrids and cultivars, under field and artificial conditions. It was found that all the accesses studied were infected by *Nectria galligena*. However, considerable differences between cultivars and hybrids in a level of damage by the disease was noted. As a result of these studies, the following cultivars and hybrids were selected as field resistant or tolerant 'Bananovoye', 'Belorusskii Sinap', 'Imant', 'Minskoye', 'Sinap Belsad', 'Belorusskoye Sladkoye', 'Pamyat Syubarovoi', 'Borovinka', 'Peppin Litovskii', 'Pepinka Zolotistaya', 'Fameuse', 'Liberty', 84-125/39, 85-2/25 and some offsprings of *M. sieboldii*. Cvs. 'Belorusskiĭ Sinap', 'Belorusskoye Sladkoye', 'Imant', 'Pamyat Syubarovoi', 'Pamyat Sikory', 'Syabryna' and the hybrid 84-125/39 proved to be very resistant to *Nectria galligena* Bres. under conditions of artificial infection. So from the plant material studied some accesses have been selected with a multiple resistance to nectria canker, scab, powdery mildew and *Phyllosticta* sp. This is very important for the future apple breeding.

## RESISTANCE OF APPLE HYBRIDS TO SCAB AND POWDERY MILDEW IN THE NURSERY

**Z.A. Kazlouskaya\*, T.A. Hashenka, and V.V. Vaseha**

The Republican Research and Production Unitary Enterprise, Institute for Fruit Growing, Samokhvalovichy, Minsk reg., 223013 Belarus  
e-mails: zoya-kozlovskaya@tut.by; tanya\_gashenko@tut.by; witalij\_waseha@tut.by

New hybrid pool was created by hybridization performed in 2006. The majority was made as backcrosses of previously selected scab-resistant seedlings that had been derived from some sources of monogenic as polygenic resistance to apple cultivar with a good fruit quality. This pool consisted of 14 progenies and 626 seedlings in total. The effects of using in the hybridization the initial forms derived from genotypes *M. × domestica* and the F<sub>1</sub>-F<sub>2</sub> *M. × zumi* and *M. sieboldii* with polygenic resistance to scab were determined in a field breeding nursery. The most successful were the cross-combinations of ‘Imant’ × 97-21/41 (‘Aborigen’ × ‘KBM F<sub>2</sub>’), ‘Imant’ × 99-9/23 (‘Utes’ × ‘BM41497’), 86-54/137 (‘Antei’ × ‘BM41497’) × ‘Pamyat Vavilova’, 86-54/137 (‘Antei’ × ‘BM41497’) × ‘Rubin’ and 25/175 (F<sub>2</sub> *M. sieboldii*) × 86-54/133 (‘Antei’ × ‘BM41497’). Combining monogenic and polygenic types of scab-resistance in one genotype allows obtaining hybrids with resistance under conditions of permanently changing race composition of the pathogen *V. inaequalis*. Scab-resistance of apple hybrid progenies was estimated under field conditions by applying the method of artificial inoculation. At least 45% of highly resistant or resistant to scab hybrid seedlings of each cross-combination was obtained. The following sources of resistance to powdery mildew were successfully included in course of hybridization: selections 21/4 *M. × zumi* and 25/175, 19/2 *M. sieboldii* as well as selections of the Belarussian apple breeding, genetically related to *M. × floribunda* 821, such as: ‘Imant’, 86-41/5 (‘BM41497’ × ‘Antei’), 99-9/42 (‘Belorusskoe Malinovoe’ × ‘Liberty’), 99-9/65 (‘Otava’ o.p.). The 11 new apple hybrids of different genetic origin were selected as donors of multiple resistance to scab and powdery mildew.



## THE HTS-BASED STUDY ON TRANSCRIPTOME OF STRAWBERRY (*Fragaria x ananassa*) INFECTED WITH *Verticillium dahliae*

M. Korbin<sup>1\*</sup>, T. Jęcz<sup>1</sup>, U. Lavi<sup>2</sup>, L. Farinelli<sup>3</sup>, M. Osteras<sup>3</sup>, N. Vincent<sup>3</sup>, and M. Dudkiewicz<sup>4</sup>

<sup>1</sup>Research Institute of Pomology and Floriculture, Skierniewice, Poland

<sup>2</sup>Institute of Plant Sciences, Agriculture Research Organisation, Volcani Research Center Bet Dagan, Israel

<sup>3</sup>Genome Analyser Service FASTERIS SA, Switzerland

<sup>4</sup>Agricultural University, Warsaw, Poland

*Verticillium* wilt caused by polyphagous fungus *Verticillium dahliae* Kleb. is considered among the most important soil-borne diseases decreasing strawberry (*Fragaria × ananassa*) production in a temperate zone. with still unknown molecular mechanism. The study on changes in strawberry transcriptome were conducted on tolerant cultivar 'Dukat' and susceptible 'Camarosa'. Plants originating from *in vitro* cultures were artificially inoculated with *V. dahliae* ( $10^7$  microconidia/ml) through wounded roots. The effectiveness of inoculation was confirmed by nested PCR (primers: VDS1/2 – D1/2) 24h after treatment. Mock-inoculated and untreated plants with uncut roots created two control levels. Total RNA was isolated from leaf and root samples that were collected one day after inoculation, according to the method described by Zeng and Yang. Use of Illumina-Solexa system (High Throughput Sequencing, HTS) for infected strawberry transcriptome analysis allowed to generate a database for leaves and roots, containing 1.8 million of unique sequences with 21 bp length. After data normalization, 60.000 TAGs were selected as the differentially expressed fragments, according to previously accepted statistical rules. Hitherto, several of one hundred primarily selected differentially expressed TAGs were identified using the NCBI GenBank data for strawberry, after their 5'RACE elongation. However, obtained data still contains huge population of the differentially expressed sequences that can be useful in further study on strawberry defense against *V. dahliae* attack, particularly when more information about *Fragaria x ananassa* genome sequence will be available for the research community.

## MAPPING OF QUANTITATIVE TRAIT LOCI FOR FIRE BLIGHTRESISTANCE IN THE APPLE CULTIVAR 'REWENA'

P-M. Le Roux<sup>1,4\*</sup>, A. Peil<sup>2</sup>, K. Richter<sup>3</sup>, C. Gessler<sup>4</sup>, and A. Patocchi<sup>1</sup>

<sup>1</sup>Plant Protection and Extension Fruits and Vegetables, Agroscope Changins Wädenswil Research Station ACW, Schloss, 8820 Wädenswil, Switzerland

<sup>2</sup>Julius Kühn-Institute, Federal Research Centre for Cultivated Plants, Institute for Breeding Research on Horticultural and Fruit Crops, Pillnitzer Platz 3a, D-01326 Dresden, Germany

<sup>3</sup>Julius Kühn-Institute, Federal Research Centre for Cultivated Plants, Institute for Resistance Research and Stress Tolerance, Erwin-Baur-Str. 27, D-06484 Quedlinburg, Germany

<sup>4</sup>Plant Pathology, IBZ, ETH Zürich, Universitätstrasse 2, 8092 Zürich, Switzerland  
e-mail: andrea.patocchi@acw.admin.ch

Fire blight, caused by the enterobacterium *Erwinia amylovora*, is a major bacterial disease of Rosaceous plants. Its damage to apple (*Malus × domestica* Borkh.) production is a concern since no existing control option has proven to be durable. Some commercial apple varieties, e.g. 'Rewena', are considered as resistant to fire blight. Thus, 'Rewena' displayed 18.5% of shoot necrosis on average after being challenged with various strains of *E. amylovora* over 11 years; in comparison, the apple cultivar 'Idared' used as susceptible control showed 78.9% of shoot necrosis in 16 years of testing (A. Peil, personal communication). In this study, we used a F1 progeny of 92 individuals derived from the cross 'Idared' × 'Rewena' to identify Quantitative Trait Loci (QTL) for fire blight resistance in 'Rewena'. Scoring of necrotic lesion lengths of the offspring after artificial inoculation with the *E. amylovora* strain Ea222 (10<sup>9</sup> cfu/ml) was performed at the Julius Kühn-Institut (Quedlinburg, Germany). Linkage maps of 'Idared' and 'Rewena' were constructed using microsatellite and DArT markers at Agroscope ACW (Wädenswil, Switzerland). Results of QTL analysis will be presented.

## OCCURRENCE OF SOOTY BLOTCH ON APPLE SELECTIONS RESISTANT TO SCAB

E. Mirzwa-Mróż<sup>1\*</sup>, M. Wińska-Krysiak<sup>2</sup>, R. Dzięcioł<sup>1</sup>, and E. Pitera<sup>3</sup>

Warsaw University of Life Sciences – SGGW, Nowoursynowska 159, 02-776 Warsaw, Poland

<sup>1</sup>Department of Plant Pathology

<sup>2</sup>Laboratory of Basic Research in Horticulture

<sup>3</sup>Department of Pomology

The study was carried out during 2006 - 2009 in the orchard of WULS-SGGW in Warsaw-Wilanów (52°9'26"N, 21°6'19"E), Mazovia province. Apple seedlings originated from *Vf* scab resistance breeding program. Crosses were performed in 1995 – 1997. Resistant seedlings were grafted on M.9 rootstock. Some tree pruning was done occasionally and none fungicides were applied. Apples with symptoms of sooty blotch were collected in the first decade of October 2006 - 2009. Immediately after harvest assessments of sooty blotch disease were done in the laboratory. The surface of the fruits covered by SBFS fungi was evaluated with six grade scale. Fungi species causing sooty blotch were identified on the base of morphology and nucleotide sequence of the rDNA internal transcribed spacer region (ITS). A total of 86 isolates were collected from fruits with visible symptoms of disease. Isolates were grown on PDA medium and identified on the base of morphological characters. DNA was extracted from representative isolates and used as matrices for PCR amplification with ITS1F and ITS4 primers. Fragments of amplified rDNA ITS were sequenced. It was found that 65% of isolates causing sooty blotch were species of genera *Microcyclosporella*, followed by *Aureobasidium pullulans* - 15%, *Microcyclospora* sp. - 17%, *Phialophora sessilis* - 1.2%, and *Peltaster fructicola* - 1.2%. Intensity of sooty blotch varied among trees, but it was no correlation between diseases level of parents and progeny.

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## GENETIC MAPPING OF THE GENE FOR SUSCEPTIBILITY TO ALTERNARIA BLOTCH IN APPLE

S. Moriya<sup>1\*</sup>, S. Terakami<sup>2</sup>, H. Iwanami<sup>1</sup>, T. Haji<sup>1</sup>, K. Okada<sup>1</sup>, T. Yamamoto<sup>2</sup>, and K. Abe<sup>1</sup>

<sup>1</sup>Apple Research Station, NARO Institute of Fruit Tree Science Shimokuriyagawa, Morioka, Iwate 020-0123, Japan

<sup>2</sup>NARO Institute of Fruit Tree Science Fujimoto, Tsukuba, Ibaraki 305-8605, Japan

Alternaria blotch, caused by *Alternaria alternata* apple pathotype, is a harmful disease of apple, particularly in East Asian countries. Although it was reported that susceptibility to Alternaria blotch was dominantly inherited, no molecular markers linked to susceptibility to this disease have been obtained. In this study, we identified *Alt* locus, which is responsible for susceptibility to Alternaria blotch in a genetic linkage map of apple, using 114 F<sub>1</sub> individuals derived from the cross between a susceptible cultivar ‘Starking Delicious’ and a resistant cultivar ‘Jonathan’. For phenotypic evaluation, five collected leaves of each F<sub>1</sub> plant were inoculated with conidial suspensions ( $5 \times 10^4$  conidia/ml) using a mist sprayer and were then incubated for 48 hours at 20°C in the dark. Mean score of each inoculated F<sub>1</sub> plant was classified as resistant or susceptible. Forty-eight plants were scored as resistant to Alternaria blotch and the other 66 were scored as susceptible. A goodness of fit was observed between this segregation and the expected segregation of 1:1 ratio ( $\chi^2 = 2.92$ ). Genome scanning approach (GSA) was used as a mapping procedure. In GSA round 1, after 22 susceptible individuals were selected and analyzed, SSR markers of three genomic regions showed significant distortion in linkage groups (LGs) 3, 10, and 11. In GSA round 2, significant linkage to *Alt* was detected only for markers located on LG 11. As a result, *Alt* was mapped between the markers Hi07d11 and DR033892 on the top of LG 11. Interestingly, *Alt* was identified in the chromosomal region homologous to *Ani* and *Ana* of the Japanese pear *Pyrus pyrifolia*, which controlled susceptibility to black spot disease caused by *A. alternata* Japanese pear pathotype. DNA markers obtained in this study will be utilized for marker-assisted selection in apple breeding programs focusing on resistance to Alternaria blotch.

## THE OCCURRENCE OF THE Highbush BLUEBERRY VIRUSES IN POLAND

E. Paduch-Cichal<sup>1\*</sup>, E. Kalinowska<sup>1</sup>, M. Chodorska<sup>1</sup>, B. Nowak<sup>3</sup>, K. Sala-Rejczak<sup>1</sup>, M. Szyndel<sup>1</sup>, Z. Gajewski<sup>3</sup>, and K. Tomala<sup>2</sup>

<sup>1</sup>Warsaw University of Life Sciences – SGGW, Faculty of Horticulture and Landscape Architecture, Department of Plant Pathology, Warsaw, Poland

<sup>2</sup>Warsaw University of Life Sciences – SGGW, Faculty of Horticulture and Landscape Architecture, Department of Pomology, Warsaw, Poland

<sup>3</sup>Faculty of Agricultural, Department of Botany and Plant Physiology, Agricultural University, Kraków, Poland

The highbush blueberry (*Vaccinium corymbosum* L.) is being infected with numerous viruses causing yield-reducing diseases. The most important viruses of the highbush blueberry are: *Blueberry shoestring virus* (BSSV), *Blueberry mottle virus* (BLMoV), *Blueberry red ringspot virus* (BRRSV), *Blueberry scorch virus* (BlScV), *Blueberry shock virus* (BlShV), *Peach rosette mosaic virus* (PRMV) and *Tobacco ringspot virus* (TRSV). A virus survey was conducted on three commercial plantations of highbush blueberry (*Vaccinium corymbosum* L.) in the central and south-east part of Poland in years 2009-2011. The plants were tested to the presence of viruses using the serological test ELISA except BRRSV, because serological detection of this virus is largely unreliable. Research material (leaves, flowers and stems) were collected from 17 highbush blueberry cultivars: Berkeley, Bluecrop, Bluejay, Bonifacy, Brigitta, Chandler, Chanticleer, Darrow, Duke, Earliblue, Herbert, Ivanhoe, Nelson, Patriot, Sierra, Spartan and Toro. The ELISA results revealed the presence in examined bushes the following viruses: *Blueberry shoestring virus* (cultivars: Bluecrop, Darrow and Herbert), *Blueberry scorch virus* (cultivars: Berkeley, Bluecrop, Bonifacy, Darrow, Duke, Earliblue, Herbert, Nelson, Patriot, Sierra and Spartan), *Tobacco ringspot virus* (cultivars: Berkeley, Bluecrop, Bluejay, Bonifacy, Brigitta, Chanticleer, Darrow, Duke, Earliblue, Herbert, Ivanhoe, Nelson, Patriot, Sierra, Spartan and Toro) and *Peach rosette mosaic virus* (cultivar Bluecrop). Reddish ring spot and blotches on stems and on the upper surface of the older leaves were noted on two cultivars: Darrow and Herbert. Symptoms were similar to those induced by *Blueberry red ringspot virus*. In order to confirm BRRSV infection by PCR technique symptomatic leaves and stems ('Darrow' - seven isolates - BRRSV03, BRRSV12, BRRSV13, BRRSV15, BRRSV16, BRRSV20, BRRSV21; 'Herbert' - two isolates - BRRSV22 and BRRSV 24) were collected. Total DNA was extracted from symptomatic leaves and stems. The primer set RRSV3/RRSV4, which amplifies the fragment of the transcriptional activator gene (Polashock et al., 2009), was used in PCR for BRRSV detection. Sequencing analysis of the fragments revealed 93-96% nucleotide sequence identity with the BRRSV-NJ putative transcriptional activator gene (GenBank Accession No. AF404509).

## EXPLORING GENOMIC ORGANISATION OF SCAB RESISTANCE IN PEAR

**D. Leforestier, C. Denancé, P. Guérif, M. Tellier, L. Bouvier, M. Chevalier, Y. Lespinasse, C.E. Durel, and L. Perchepped\***

Genetics and Horticulture – GenHort, UMR1259 INRA/ACO/UA, INRA Centre d'Angers-Nantes, 42 rue G. Morel, BP 60057, 49071 – Beaucouzé cedex, France

Pear scab, caused by the fungus *Venturia pirina*, is the most important disease of European pear. Most of the grown European pear cultivars being susceptible to scab, numerous chemical treatments are necessary to control scab in commercial orchards. Working on scab resistance will thus contribute to the reduction of pesticide inputs. Genetic markers and genomic approaches should help the creation of new pear varieties resistant to scab. Numerous scab resistance genes and QTLs have already been mapped in apple against *Venturia inaequalis*. In Asian and European pears, only 3 major genes and two QTLs have been identified and mapped up to now. Colocalisation of the last resistance gene, *Rvp1*, with a cluster of scab resistance genes and QTLs on the linkage group 2 of the apple genome underlines the expected synteny between pear and apple for at least some of the scab resistance factors. Genetic mapping of resistance factors in two new pear scab resistance sources (P3480 and Euras) is underway. First, phenotyping of two segregating progenies ('Angély × P3480' and 'Euras × P3295') was done twice in 2010 and 2011. Then, genotyping of the progenies was preferentially engaged in genomic regions known to carry scab resistance factors in apple using SSR markers. Two genomic regions were shown to carry pear scab resistance factors on linkage groups 1 and 4. More accurate mapping of the involved genomic regions is in progress. Genetic basis of scab resistance in pear and genomic organisation of these resistance loci in comparison with apple will be further explored with the help of transferable genetic markers and whole genome sequence already available for apple and in progress for pear.

## DEVELOPMENT OF A GENETIC LINKAGE MAP FOR IDENTIFICATION OF MOLECULAR MARKERS ASSOCIATED WITH RESISTANCE TO BACTERIAL SPOT (*Xanthomonas arboricola* pv. *pruni*) IN PEACH

N. Yang<sup>1</sup>, G. Reighard<sup>1\*</sup>, D. Ritchie<sup>2</sup>, W. Okie<sup>3</sup>, and K. Gasic<sup>1</sup>

<sup>1</sup>Department of Environmental Horticulture, Clemson University, Clemson, SC 29634, USA

<sup>2</sup>Department of Plant Pathology, CB 7616 North Carolina State University, Raleigh, NC 27695 USA; <sup>3</sup>ARS-USDA, S.E. Fruit & Tree Nut Research Lab, Byron, GA 31008 USA

Bacterial spot, caused by *Xanthomonas campestris* pv. *pruni*, is a serious disease that can affect peach fruit quality and production worldwide. This disease causes severe defoliation and blemishing of fruit, particularly in areas with high rainfall, strong winds, high humidity and sandy soil. The molecular basis of its tolerance and susceptibility in peach is yet to be understood. To study the genetics of the peach in response to bacterial spot, a segregating population between two peach cultivars, Clayton, a resistant phenotype, and O'Henry, which is very susceptible to bacterial spot, was created. The F<sub>2</sub> population was planted at three locations: the Sandhills Research Station, Jackson Springs, North Carolina (NC); the Sandhill Research and Education Center, Pontiac, South Carolina (SC); and the ARS-USDA Southeastern Fruit and Tree Nut Laboratory at Byron, Georgia (GA). Field data for leaf and fruit response to bacterial spot infection were collected at two locations, NC and SC during two seasons. Out of 574 SSR markers developed in *Prunus* and mapped in peach, only 7% (38) were informative among parents and used to develop a framework genetic linkage map. High broad sense heritability, obtained from the phenotypic data, indicated that major genes are responsible for bacterial spot resistance in peach and suggested involvement of different genes in leaf and fruit resistance. Single marker analysis, Kruskal & Wallis, and Simple Interval Mapping revealed one putative QTL associated with low incidence of bacterial spot located on LG4. An effort to increase the map density around putative QTL locations using genomic DNA sequences sharing homology with the NBS-LRR (nucleotide binding site, leucine-rich repeat) resistance genes is ongoing. Marker-assisted selection for bacterial spot resistance will be discussed.

## ***Malus* sp. – *V. inaequalis* INTERACTION CHARACTERISTICS AMONG ACCESSIONS OF APPLE GENETIC RESOURCES**

**S. Sikorskaite\*, D. Gelvonauskiene, V. Bendokas, V. Stanys, and D. Baniulis**

Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry, Babtai LT-54333, Kaunas reg., Lithuania

Apple fruit production is a leading horticultural practice in Lithuania. Apple scab is one of the most damaging apple tree diseases that cause additional costs and ecological risks due to plant protection practices. Therefore identification of new sources of apple scab resistance genes and efficient introduction of the genes to apple cultivars is crucial for apple breeding. Apple trees have been grown in the Northern Europe for centuries, and a number of traditional cultivars carrying genes for qualitative and quantitative resistance are spread in the region. However, pedigree of many of the traditional cultivars is unknown, and information on genetic background of resistance of the cultivars is scarce. Over 40 accessions of the traditional cultivars and cultivars derived from crosses with the traditional cultivars are deposited at the collection of genetic resources of the Institute of Horticulture, LRCAF. In this study we classified morphological traits of apple scab resistance of 37 traditional cultivars and cultivars released at the Institute during the last century. Complete resistance (class 0) was observed only in cultivars carrying *Vf* resistance gene. Five cultivars lacked resistance and demonstrated extensive sporulation symptoms (class 3b-4). Ten cultivars exhibited resistance with chlorosis and necrosis symptoms without sporulation (class 2) or scarce sporulation levels (class 2-3a). The remaining cultivars displayed low level of resistance with varying degree of sporulation symptoms (class 3a-3b). Analysis of genetic polymorphism of microsatellite loci established genetic relationship among the traditional cultivars and 10 reference cultivars used in the analysis. Putative links between genetic background of the cultivars and identified resistance phenotypes were established.



## MAPPING QTL RESISTANCE AGAINST *Xanthomonas arboricola* pv. *pruni* IN APRICOT

**D. Socquet-Juglard<sup>1,3\*</sup>, D. Christen<sup>2</sup>, B. Duffy<sup>1</sup>, C. Gessler<sup>3</sup>, and A. Patocchi<sup>1</sup>**

<sup>1</sup>Agroscope Changins-Wädenswil ACW, Plant Protection Division, CH-8820 Wädenswil, Switzerland

<sup>2</sup>Agroscope Changins-Wädenswil ACW, Conthey Research center, 1964 Conthey, Switzerland

<sup>3</sup>ETH Zurich, Institute of Integrative Biology, Plant Pathology, 8092 Zurich

*Xanthomonas arboricola* pv. *pruni* (Xap) is the causal agent of bacterial spot of stone fruits. It is one of the major diseases of peach, apricot, plum and almond, and is regulated as a quarantine pathogen in Europe. The pathogen causes necrotic lesions on fruits and leaves; it renders fruits unmarketable and in extreme years severe defoliation may lead to tree death. To identify quantitative trait loci (QTLs) associated with Xap resistance, we developed an F1 population from a cross between the Canadian variety Harostar (resistant) and the French variety Rouge de Mauves (susceptible). A population of 100 genotypes was obtained and for each parent a genetic linkage map has been developed with 104 microsatellite markers and 5 AFLP primer combinations yielding on average 65 AFLP-markers per parent. Different inoculation techniques were tested and dipping the plant into the inoculum was found to be the most efficient way to discriminate resistance levels among genotypes. Five replicates per genotype were inoculated with a mix of four Xap strains and were scored 14, 30 and 45 days post inoculation for disease incidence, for the average spot number per leaf and for the average of damaged surface per leaf. AUDPCs were calculated for each of these traits. Inoculation has been repeated in 2011 to check the stability and the accuracy of the QTLs over 2 years. The results of the ongoing analysis will be presented.

## **INHERITANCE OF ANTHRACNOSE (*Sphaceloma ampelinum*) RESISTANCE IN GRAPEVINE**

**P. Tantasawat\*, O. Poolsawat, and S. Wongkaew**

School of Crop Production Technology, Suranaree University of Technology, 111 University Avenue, Muang District, Nakhon Ratchasima 30000, Thailand

Anthraco­nose is one of the major diseases of most grapevine cultivars grown in Thailand. Therefore, breeding for improved anthracnose resistance is crucial. Six factorial crosses were made between three anthracnose and downy mildew resistant hybrids (NY88.0517.01, NY65.0550.04 and NY65.0551.05; male parents) and two susceptible cultivars of *Vitis vinifera* L. ('Black Queen' and 'Carolina Black Rose', female parents). Eighty-five seedlings were evaluated for anthracnose resistance. The General Combining Ability (GCA) variance among male parents was significant while the variance of GCA among females and Specific Combining Ability (SCA) variance were not significant, indicating the prevalence of additive over non-additive gene actions. The estimated narrow sense heritability of anthracnose resistance was 79.2%, suggesting that anthracnose resistance gene(s) were highly heritable. The 'Carolina Black Rose' × NY65.0550.04 and 'Black Queen' × NY88.0517.01 crosses with the highest proportions of resistant seedlings are recommended for future use.

## EVALUATION OF LOCAL APPLE VARIETIES IN NORTHERN ITALY AS SOURCES OF SCAB RESISTANCE

M. Komjanc<sup>1</sup>, E. Zini<sup>1\*</sup>, M. Troglio<sup>1</sup>, R. Osler<sup>2</sup>, R. Velasco<sup>1</sup>, and P. Ermacora<sup>2</sup>

<sup>1</sup>Dipartimento Genomica e Biologia delle Piante da Frutto, Fondazione Edmund Mach, Via Edmund Mach 1, 38010 San Michele All'Adige (TN) Italy

<sup>2</sup>Dipartimento di Biologia applicata alla Difesa delle Piante, Università di Udine, Via delle Scienze 208, 33100 Udine (UD) Italy

Management strategies for apple scab control are based on intensive chemical treatments or by using scab resistant genotypes, mainly containing *Vf* gene derived from *Malus floribunda*. Unfortunately this monogenic resistance has been overcome, in some cases, by new races of the pathogen and at the same time, organoleptic properties of some *Vf* containing varieties are not always well accepted from the customers. Some apple genotypes, belonging to germplasm collection established in Friuli Venezia Giulia region, have shown characteristics of resistance to apple scab both in field than in artificial infection conditions. In particular, three local varieties (Striato Dolce, Dal Dolç, Di Coròne) demonstrated Mendelian inheritance under single gene control after scab infection. In this work, we focused on the progeny derived from a cross between Striato Dolce and Golden Delicious. Striato Dolce is a local variety of the Carnia region; mother plant is about eighty years old and is located in Arta Terme (Friuli Venezia Giulia). The geographic origin is still unknown, some witnesses would go back to the trade inside the Austro-Hungarian empire. An Illumina bead array of 384 SNPs, based on the GoldenGate assay was developed from a set of 1,679 SNPs mapped in an integrated apple genetic map (<http://genomics.research.iasma.it/>). The 384 SNPs were selected to be evenly spaced throughout the 17 apple linkage groups. The population maps were created with 327 SNP markers. The resistance to apple scab, derived from the autochthonous variety, was confirmed to be monogenic after two years of evaluation in greenhouse. The putative resistance gene was localized in linkage group 9, revealing that the source for this type of resistance is different from all scab resistance genes identified until now.



**Poster session**

**MANAGEMENT AND RESEARCH ON GENETIC  
RESOURCES (PART 2)**



## GENETIC DIVERSITY BETWEEN PEAR CULTIVARS IN IRAN USING SIMPLE SEQUENCE REPEATS MARKER (SSR)

**J. Erfani<sup>1</sup>, H. Abdollahi<sup>2</sup>, A. Ebadi<sup>1</sup>, R. Fattahi<sup>1</sup>, M. Khorramdelazad<sup>3</sup>, and F. Babaei<sup>3\*</sup>**

<sup>1</sup>Horticulture Department, Campus of Agricultural Faculty, University of Tehran, Karaj, Iran

<sup>2</sup>Horticulture Department, Seed and Plant Improvement Research Institute, Karaj, Iran

<sup>3</sup>Faculty of Agriculture, Azad University, Science and Research Unit, Tehran, Iran

The development of highly informative DNA marker is essential for genetic studies and marker-assisted selection of agronomically important traits in pear. Simple sequence repeat (SSR) markers were used to assess genetic diversity and relationship of *Pyrus L.* cultivars in Iran. The genetic variability and relationships among forty seven pear genotypes (including 4 Japanese pear, 41 European pear as well as 2 wild relatives) were achieved using 28 microsatellites primers pair's combinations. The level of polymorphism for all genotypes as revealed by SSR marker was 76.3% and the size of amplified fragments ranged between 81 and 245 bp. A total of 174 allelic variants were detected at 28 SSR loci, ranging from three to 12 per locus with an average of 6.21. The entire microsatellites marker to amplify more than three loci in some of the genotypes studied. The Heterozygosity Observation ( $H_o$ ) values of the loci ranged from 0.31 (TsuENH006F) to 0.72 (NB103a). The Dice's similarity coefficient between cultivars with SSR data ranged from 30% (two genotypes SK9 and *P. mazandaranica*) to 93% (two genotypes SK7 and SK9). UPGMA cluster analysis of similarity all the genotypes were obtained, showing 2 major groups corresponding to the Japanese and European pear. The use of 28 SSR markers was sufficient to differentiate between all of the 47 genotypes.

## STUDY OF GENETIC DIVERSITY OF SELECTED IRANIAN ALMOND GERMPLASMS USING FLUORESCENT-AFLP MARKERS

M. Farsi<sup>1,2\*</sup>, M. Zeinalabedini<sup>1</sup>, A. Imani<sup>3</sup>, B. Nakhoda<sup>4</sup>, P. Majidian<sup>1,5</sup>, M. Dabbab<sup>1,6</sup>, and M. Mardi<sup>1</sup>

<sup>1</sup>Department of Genomics, Agricultural Biotechnology Research Institute of Iran, Karaj, Iran

<sup>2</sup>Department of Plant Breeding, Karaj Branch, Islamic Azad University, Iran

<sup>3</sup>Horticultural Department, Seed and Plant Improvement Institute, Karaj, Iran

<sup>4</sup>Department of Systems Biology, Agricultural Biotechnology Research Institute of Iran, Karaj, Iran

<sup>5</sup>Department of Agronomy and Plant Breeding, Faculty of Agriculture and Natural Resources of Sari, Iran

<sup>6</sup>Department of Agronomy and Plant Breeding, Faculty of Agriculture and Natural Resources, Islamic Azad University, Science and Research Branch, Tehran, Iran

Almond (*Prunus dulcis* L.) is an old nut and fruit tree. Middle East region is considered as center of origin of this species. Due to suitable climatic conditions, Iran is one of the most important growing centers for different varieties of almond. Iran ranks third after US and Spain for almond annual production. Because of its self-incompatible nature that requires cross-pollination for seed production, and its vegetative propagation as a crop, the almond has maintained a high level of genetic variability. Here, we examined genetic diversity of 50 new almond genotypes collected from Iran, using fluorescent-AFLP markers. Five pairs of primers showed distinct patterns of polymorphic bands among 25 pairs of primers. The results demonstrated high level of polymorphism in almond genotypes. Phylogenetic analysis using split-decomposition method showed that all the genotypes under study can be categorized in different distinct clusters based on their origin. Aside from the capability of this technique for phylogenetic studies, it can be used for evaluating genetic diversity and relationships, documentation of Iranian almond germplasms, and establishing a core collection. The outputs can ultimately be used as helpful and complementary tools for almond germplasm management and breeding programs.



## ANALYSIS LINKED SSR MARKERS ASSOCIATED TO GENE CONTROLLING OF TRAITS IN QUINCE (*Cydonia oblonga* Mill.)

M. Khorramdelazad<sup>1\*</sup>, H. Abdollahi<sup>2</sup>, and M. Alipour<sup>1</sup>

<sup>1</sup>Faculty of Agriculture, Azad University, Science and Research Unit, Tehran, Iran

<sup>2</sup>Horticulture Research Department, Seed and Plant Improvement Research Institute, Karaj, Iran

Low information is available on gene controlling of important traits in quinces. In order to study the linked SSR Markers associated to the genetic controlling of morphological traits, cross-genus application of SSR markers developed in apple and pear was examined by 15 primer pairs contain 9 from apple and 6 from pear on quince genotypes. Morphological assessment started at the same time on 30 important morphological traits based on UPOV descriptor on 28 out of 45 quince genotypes from National Quince Collection of Iran. SSR data showed totally 80 amplified alleles by average 6 alleles per locus. 15 alleles out of 80 amplified alleles had high correlation to the morphological traits, including 2 very high ( $P > 99.99\%$ ) and 13 high ( $P > 99\%$ ) correlated alleles. Also 65% of correlations were related to the alleles amplified by apple SSR primers. The most associated morphological traits to the SSR alleles were belong to the fruits, blooms, leaves and tree, respectively with 6, 4, 3 and 1 associated relationship. In addition, 90% of associated traits were quantitative, including two very high trait/allele correlations. The most trait/allele associations were allele III and V from CH04e03 locus respectively with positive and negative correlations to the stalk basin, followed by CH50d04, CH04e03 and NB103a with fruit length, fruit diameter, fruit weight and forms of stalk and eye basin of the fruits.

## IDENTIFICATION OF SYNONYMS BY OLD HUNGARIAN APPLE CULTIVARS USING MORPHOLOGICAL AND MOLECULAR MARKERS

I. Király<sup>1\*</sup>, O. Nagystván<sup>1</sup>, É.S. Erdélyi<sup>2</sup>, and M. Tóth<sup>1</sup>

<sup>1</sup>Corvinus University of Budapest, Faculty of Horticultural Science, Department of Pomology, Villányi út 29-43, H-1118 Budapest, Hungary

<sup>2</sup>Corvinus University of Budapest, Faculty of Horticultural Science, Department of Mathematics and Informatics

e-mail: ildiko.kiraly@uni-corvinus.hu

In case of old apple cultivars it is usual that one cultivar has several different names or more cultivars have the same name. We examined some - in the relevant literatures submitted - synonym cultivars of 'Cigány alma' and 'Húsvéti rozmaring' to verify the relationship. There are several hundred cultivars within the group of Renet apple cultivars. We analysed those 10 Renet cultivars which can be found in our apple collection. In the present study twelve SSR loci were selected that produced considerable polymorphisms in previous reports to verify and distinguish synonyms, homonyms and cultivar groups of old Hungarian apple cultivars. 12 applied primer pairs were CH01f02, CH01h01, CH02c02a, CH02c09, CH05e03, CH04e03, CH05d11, CH03g07, CH02c11, CH02d08, CH03a02 and CH05c04. Cultivars were also characterised by morphological description with the code numbers of the UPOV. Differences have been found between 'Cigány alma' and 'Simonffy piros' with morphological and SSR markers, so they are not synonyms. The examined Rozmaring cultivars could not be distinguished from each other neither with morphological nor with the 12 SSR markers, so they are synonyms. The applied morphological and molecular SSR investigations were both suitable to distinguish the tested Renet cultivars from each other.

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## SSR MARKER BASED FINGERPRINTING FOR SOUR CHERRY (*Prunus cerasus*) GENETIC RESOURCES IDENTIFICATION AND MANAGEMENT

G. Lācis\* and I. Kota

Latvia State Institute of Fruit-Growing, Graudu Str.1, Dobele LV-3701, Latvia  
e-mail: gunars.lacis@lvai.lv

The genetic diversity of sour cherry (*Prunus cerasus* L.) germplasm and relationships among accessions in the collection is crucial for development of plant material maintenance strategy. Fifty sour cherry accessions maintained at the genetic resources collection of the Latvia State Institute of Fruit-Growing (LIFG), Dobele were evaluated using 26 SSR markers. Two sets of SSR markers have been applied: sixteen markers approved by ECPGR *Prunus* WG and the set of ten markers applied until now at the LIFG. Successful amplification was observed for 25 tested markers (except for marker PS05C03), whereas marker EMPA026 showed monomorphic band pattern in the tested germplasm. Other tested SSR markers were highly polymorphic with 5 - 20 alleles per locus. Heterozygosity and gene diversity values ranged from 0.520 to 1.000 and 0.539 - 0.865, respectively, while discrimination power per locus varied from 0.307 to 0.918. Four to 27 genotypes were found in analysed sour cherry collection for the 25 tested SSR markers. Higher average values of allele number, observed heterozygosity as well as gene diversity were observed for LIFG marker set, however average discrimination power was identical for both marker sets. Especial interest in the evaluation of genetic diversity was paid to the clones of landrace 'Latvijas Zemais', which is the most important cultivar in the Latvian sour cherry production. In total 25 clones collected throughout the country were analysed to evaluate their genetic relatedness as well as for detection of identity. Two genetically identical clones of cv. 'Latvijas Zemais' were found, that should be taken into account in the maintenance strategy. In general, clones of cv. 'Latvijas Zemais' showed high mutual genetic similarity and distinction from other Latvian sour cherry cultivars as well as cultivars developed in other countries.

## GENETIC VARIABILITY AND RELATIONSHIP OF THE HERITAGE PEACH VARIETIES OF EMILIA ROMAGNA (ITALY)

A. Leone<sup>1\*</sup>, M.T. Dettori<sup>2</sup>, A. Liverani<sup>1</sup>, S. Sirri<sup>1</sup>, I. Verde<sup>2</sup>, and D. Giovannini<sup>1</sup>

<sup>1</sup>Unità di Ricerca per la Frutticoltura di Forlì (CRA-FRF), Via la Canapona, 1 bis, 47121 Forlì, Italy

<sup>2</sup>Centro di Ricerca per la Frutticoltura di Roma (CRA-FRU), Via Fioranello, 52, 00134 - Roma, Italy

Traditional peach (*Prunus persica* (L.) Batsch) varieties of Emilia Romagna (southern Po valley, Italy) territory are mostly characterized by melting freestone, juicy, aromatic white-fleshed fruit, susceptible to bruising and short shelf-lived. By the end of World war II, these varieties were rapidly abandoned in favor of the yellow-fleshed peaches from USA breeding programs, less susceptible to manipulation and more suited to market demand. The Fruit Tree Research Unit of Forlì of the Agricultural Research Council (CRA-FRF) has recently undertaken the collection and characterization of heritage peaches of Romagna, with the aim to preserve from erosion its valuable agronomic traits. In order to rationalize the collection, to detect synonyms and homonyms, to explore the genetic diversity and relatedness of the traditional peach genetic resources, twenty-six peach accessions were fingerprinted with 16 microsatellite (SSR) primer pairs, chosen because highly polymorphic and well-spaced along the 8 linkage groups of the *Prunus* T × E reference map. Genomic DNA, extracted from young leaves, was amplified by using the chosen SSR primers; amplicons obtained were ran by PAGE (Polyacrylamide gel electrophoresis) method and silver stained. The size of each fragment was scored using three different size markers: to elucidate both genetic relationships and genetic diversity among accessions, data were analyzed and number of alleles/locus, allele frequency, observed heterozygosity and the similarity index among the accessions were calculated. Nineteen accessions showed a unique fingerprint. Despite being selected in a restrict territory, heritage peaches of Romagna showed a high heterozygosity and high number of rare alleles, which is indicative of their potential genetic richness and benefits to future breeding programs.

## CHARACTERIZATION OF MICROSATELLITE MARKER LOCI IN LITHUANIAN PEAR (*Pyrus communis*) CULTIVARS

**V. Lukoseviciute\***, D. Gelvonauskiene, G. Staniene, D. Baniulis, A. Sasnauskas, V. Stanys, and R. Rugienius

Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry, Babtai LT-54333, Kaunas reg., Lithuania

Genus *Pyrus* includes species and cultivars of great genetic diversity. However, genetic resources of *Pyrus* have not been fully characterised due to widespread crossability among species and lack of differentiating morphological markers. Therefore, tools for assessment of genetic polymorphism and genotyping are required for breeding and research on pear genetic resources. Polymorphic microsatellite loci are the markers of choice in genetics and breeding studies due to their multi-allelic nature, codominant inheritance, high abundance, reproducibility, transferability over genotypes and extensive genome coverage. In this study, microsatellite loci suitable for genotyping and genome mapping of Lithuanian origin and local traditional pear cultivars were characterised. The screen included 18 traditional Lithuania pear cultivars and 27 common pear cultivars adapted to local climatic conditions. In a multiplex analysis setup, consistent results were obtained with 8 primer pairs. The number of alleles per locus ranged from 8 to 18 (11.7 on average). The most polymorphic microsatellite loci among the 45 pear cultivars were EMPc106 (PIC value 0.89), EMPc117 and NB109a (PIC value 0.82). The set of markers sufficient to distinguish all tested Lithuanian and standard pear cultivars was identified. Analysis of genetic polymorphism of microsatellite loci established genetic relationship among the traditional cultivars. Putative links to known pedigrees and sites of origin were assessed.

## DNA FINGERPRINTING OF NEW IRANIAN APRICOTS RELEASED FROM BREEDING PROGRAMS

**M. Dabbab<sup>1,2</sup>, M. Zeinalabedini<sup>1</sup>, J. Dejampour<sup>3</sup>, P. Majidian<sup>1,4\*</sup>, B. Nakhoda<sup>5</sup>, M. Farsi<sup>1,6</sup>, M. Mardi<sup>1</sup>, and A. Imani<sup>7</sup>**

<sup>1</sup>Department of Genomics, Agricultural Biotechnology Research Institute of Iran, Mahdasht Rd., 31535-1897, Karaj, Iran

<sup>2</sup>Department Of Agronomy and Plant Breeding, Faculty of Agriculture and Natural Resources, Islamic Azad University, Science and Research Branch, Tehran, Iran

<sup>3</sup>Agriculture Research Center of East-Azerbaijan, Tabriz, Iran

<sup>4</sup>Department of Agronomy and Plant Breeding, Faculty of Agriculture and Natural Resources of Sari

<sup>5</sup>Department of Systems Biology, Agricultural Biotechnology Research Institute of Iran, Mahdasht Rd., 31535-1897, Karaj, Iran

<sup>6</sup>Department of Plant Breeding, Karaj Branch, Islamic Azad University

<sup>7</sup>Horticultural Department, Seed and Plant Improvement Institute, Karaj, Iran

Apricot (*Prunus armeniaca* L.) is one of the most important *Prunus* species. Iran is the second producer of apricot in the world. Due to its high level of heterozygosity and genetic diversity as well as long time to reach reproductive stage, it is so hard to determine their genetic authenticity before maturity. For genetic authenticity, different assessments such as morpho-pomological traits, biochemical, and molecular markers are available. In this study we use a collection of 44 apricot genotypes from Iranian apricot breeding program. Thirty fluorescent-AFLP primer pairs were screened to characterize the new cultivars and establish their genetic relationships and fingerprints; out of those, five primers clearly amplified repeatable polymorphic patterns, which precisely recognized all the genotypes. We found out unique and informative banding patterns of fluorescent-AFLP markers along with morpho-pomological characteristics can be used as powerful tools to distinguish new genotypes and identification of their genetic authenticity. Aside from that, this technique is much faster and cost effective, and can be used even at seedling stage.

## EVALUATION OF GENETIC RELATIONSHIPS OF SOME APRICOT CULTIVARS AND GENOTYPES USING FLUORESCENT-AFLP MARKERS

**P. Majidian<sup>1,2\*</sup>, M. Zeinalabedini<sup>1</sup>, J. Dejampour<sup>3</sup>, B. Krska<sup>4</sup>, B. Nakhoda<sup>5</sup>, M. Dabbab<sup>1,6</sup>, M. Farsi<sup>1,7</sup>, M. Mardi<sup>1</sup>, and A. Imani<sup>8</sup>**

<sup>1</sup>Department of Genomics, Agricultural Biotechnology Research Institute of Iran, Mahdasht Rd., 31535-1897, Karaj-Iran

<sup>2</sup>Department of Agronomy and Plant Breeding, Faculty of Agriculture and Natural Resources of Sari

<sup>3</sup>Agriculture Research Center of East-Azerbaijan, Tabriz-Iran

<sup>4</sup>Mendel University, Berno Horticulture Faculty in Lednice, Czech Republic

<sup>5</sup>Department of Systems Biology, Agricultural Biotechnology Research Institute of Iran, Mahdasht Rd., 31535-1897, Karaj-Iran

<sup>6</sup>Department of Agronomy and Plant Breeding, Faculty of Agriculture and Natural Resources, Islamic Azad University, Science and Research Branch, Tehran, Iran

<sup>7</sup>Department of Plant Breeding, Karaj Branch, Islamic Azad University

<sup>8</sup>Horticultural Department, Seed and Plant Improvement Institute, Karaj, Iran

Apricot (*Prunus armenica* L.), a fruit species of the family *Rosaceae*, genus *Prunus* L., is widely distributed in the Mediterranean region and the Middle East, especially in Iran. Vast diversity among apricot cultivars and genotypes has been observed in Iran, which is a very good genetic resource for breeding programs. Suitable climatic conditions for apricot production and its economic importance were the main reasons for its vast area under cultivation and diversity. We studied genetic diversity of 42 apricot cultivars and genotypes collected from new Iranian and Czech Republic collections using fluorescent-AFLP markers. The objectives of this study were to determine the genetic structure and genotypic diversity among different eco-geographical populations. We observed that five pairs of fluorescent-AFLP primers showed clear banding patterns and high level of polymorphism amongst the 30 pairs of primers screened. Genetic structure of different genotypes and their relationships assessed by clustering-based method analysis using phylogenetic network and model-based method. Different groups of cultivars and genotypes were detected based on their origin and geographical distribution. Our findings showed that in comparison with traditional AFLP, fluorescent-AFLP markers are more efficient in evaluating genetic diversity and population structure among apricot genotypes and even other *Prunus* fruit tree species.

## THE SURVEY OF GENETIC DIVERSITY & POPULATION STRUCTURE ANALYSIS OF IRANIAN SWEET POMEGRANATE (*Punica granatum* L.) GERMPLASM USING SSR MARKERS

S.M. Mousavi Derazmahalleh<sup>1\*</sup>, M. Mardi<sup>2</sup>, S.H. Marashi<sup>3</sup>, S. Malekzadeh<sup>3</sup>, S.M. Khayam Nekoui<sup>2</sup>, M. Zeynalabedini<sup>2</sup>, M. Kazemi<sup>4</sup>, and T. Roodbar Shojaie<sup>4</sup>

<sup>1</sup>Ferdowsi university of Mashhad

<sup>2</sup>Agricultural Biotechnology Research Institute of Iran (ABRII), Genomics Department, road Mahdasht, Karaj

<sup>3</sup>Ferdowsi University of Mashhad, Agricultural Department

<sup>4</sup>Agricultural Biotechnology Research Institute of Iran (ABRII), Genomics Department

The pomegranate (*Punica granatum* L.) is native horticultural plants of Iran which have been cultivated from ancient times for its economic, ornamental and medicinal properties globally. Although the number of *Punica* species is very low, but there is a high morphological diversity have been observed within the varieties and genotypes. In this study, seven microsatellite markers (SSR) were tested on 194 samples of the sweet pomegranate germplasm. All primers have shown significant polymorphism among the studied samples. Total numbers of observed alleles were 29, the number of alleles per locus have ranged between 2 to 8. Average polymorphism information content and heterozygosity of these primers were 0.807 and 0.847 respectively, have proved strong nature of microsatellite markers in genetic diversity studies. In order to assess of genetic relationships and population structure, Cluster analysis was performed by methods UPGMA and NJ with Mega4 software, structure analysis model-based Bayesian by Structure 2.2 software and principal coordinate analysis (PCoA) with Ntsys software. Results of all mentioned methods have shown that cultivars and genotypes are generally clustered independently from their geographical origin and their proposed denomination suggesting that severe admixture in studied samples. Survey of our study indicated that there is a high genetic diversity among of sweet pomegranate germplasm in Iran.



## THE STUDY OF GENETIC DIVERSITY AND RELATIONSHIPS AMONG SOME WILD *Prunus* SPECIES USING VEGETATIVE TRAITS AND MOLECULAR MARKERS

**K. Nikoumanesh\*, A. Ebadi, and M.R. Fattahi**

Department of horticulture, College of Agriculture, University of Tehran, Karaj, Iran  
e-mail: nikou@alumni.ut.ac.ir

Iran due to its suitable climatic conditions is one of the main growing regions for commercial and wild almonds. In this study genetic diversity and relationships among seven *Prunus* species belonging to subgenus *Amygdalus* (*P. scoparia*, *P. arabica*, *P. eburnea*, *P. erioclada*, *P. lycioides*, *P. orientalis* and *P. communis*) were investigated at morphological and molecular levels. 15 vegetative traits which are important for rootstock improvement were evaluated during two growing seasons. Principle component analysis divided all the traits into four main components which explained 87.6% of total variation. Morphological clustering using Ward's method divided all the samples into four main groups while the molecular UPGMA dendrogram divided them into three main groups. The maximum number of band was generated by primer BB5 (26 fragments) and the minimum was generated by primers BA17, BD9 and BE18 (12 fragments). Also the maximum and minimum values of Resolving Power were generated by BD10 and BB16 respectively. In molecular analysis based on Jaccard's similarity coefficient the minimum value of similarity was observed between *P. lycioides*-T and *P. communis* (0.25) whereas the maximum value of similarity was observed between *P. scoparia* N-1 and *P. scoparia* ES (0.71). Our results indicated an extensive genetic diversity for almond rootstock improvement in Iran.

## **MORE HARMONIZATION NEEDED FOR DNA-BASED IDENTIFICATION OF FRUIT TREE GERMPLASM**

**J. Sehic, L. Garkava-Gustavsson, and H. Nybom\***

Department of Plant Breeding and Biotechnology – Balsgård, Swedish University of Agricultural Sciences, SE-29194 Kristianstad, Sweden

Considerable efforts have been made to provide fruit tree and *Vitis* genebank accessions with DNA-based identification profiles. Recent reviews show that number of mislabelled accessions is much higher when, e.g., simple sequence repeats (SSR) are used (typically 25 – 30% mislabellings) instead of the traditional pomological or ampelographic characters (typically 5 – 10%). Obvious problems are revealed when finding the same DNA profile for accessions with different names, and different DNA profiles for accessions with the same name. Number of mislabellings may be even higher since finding a unique DNA profile with a unique cultivar name, does not necessarily mean that the name on the label is the correct one. Unless comparisons can be made across other gene banks, it is often difficult to know if the material is correctly identified, and whether it truly represents a unique, domestically evolved cultivar or imported material that has just been renamed. In our recent SSR-based analyses of apple (*Malus domestica*), European pear (*Pyrus communis*), sweet cherry (*Prunus avis*) and sour cherry (*Prunus cerasus*) cultivars in the Swedish gene bank system for mandate cultivars, about 30% were definitely mislabelled. For some of the *Pyrus* samples, DNA profiles could be compared to data from Brogdale, since they had used the same set of SSR loci recommended by ECPGR. Similarly, some of the SSR loci used in apple have been shared by several research groups. In most cases it is, however, impossible to make valid comparisons across studies since different sets of loci are used in different laboratories. This is a major problem, seriously undermining research and breeding based on access to correctly identified gene bank material. Here, we will present a compilation of the SSR loci used in major SSR-based screenings of apple, pear and cherry germplasm– and also discuss other DNA-based methods that may prove more efficient.

## GENETIC IDENTIFICATION OF 'BISTRICA' AND HER SYNONYMS 'POŽEGAČA' AND 'HAUSZWETSCHÉ' (*Prunus domestica* L.) USING SSRs

D. Halapija Kazija<sup>1</sup>, P. Drkenda<sup>2</sup>, S. Šimon<sup>3</sup>, M. Žulj<sup>3</sup>, F. Gaši<sup>2</sup>, D. Nikolić<sup>4</sup>, P. Vujević<sup>1</sup>, T. Jelačić<sup>1</sup>, B. Milinović<sup>1</sup>, M. Kurtović<sup>2</sup>, and I. Pejić<sup>3\*</sup>

<sup>1</sup>Croatian Centre for Agriculture, Food and Rural Affairs, Institute of Viticulture, Enology and Pomology, Jandrićeva 42, HR-10000 Zagreb, Croatia

<sup>2</sup>Faculty of Agriculture and Food Sciences, University Sarajevo, Zmaja od Bosne 8, 71000 Sarajevo, B&H

<sup>3</sup>University of Zagreb Faculty of Agriculture, Svetošimunska 25, HR-10000 Zagreb, Croatia

<sup>4</sup>University of Belgrade Faculty of Agriculture, 6 Nemanjina Str., 11080 Zemun, Serbia

'Bistrica' is well known and widespread plum variety in the area of former Austro-Hungarian Empire being under cultivation for more than 300 years. Although 'Bistrica' is well known and described variety, due to often observed phenotypic variation it has been reported as "landrace cultivar" consisted out of many different "biotypes". Thus, reliable genotype identification *in situ* in old traditional orchards can be difficult. The aim of this study was to determine its SSR profile and to estimate its genotype homogeneity across the region. Therefore 33 accessions of 'Bistrica' and her synonyms were sampled in both traditional orchards and nurseries from Bosnia & Herzegovina, Croatia and Serbia. A known synonym cultivar, originating from Austria ('Hauszwetsche'), was used as a referent genotype too. Set of 11 highly polymorphic SSR markers was used for molecular characterization and PCR products were detected using ABI 3130 Genetic Analyzer (Applied Biosystems). Three accessions out of 33 had a genotype different at most of the analyzed loci and we excluded them from further consideration as homonyms. Nineteen out of 33 accessions shared identical genotype at 11 SSR loci having 4 - 6 alleles per locus. The origin of these accessions was partly from nurseries (10 samples from three different nurseries in Croatia and Serbia) and partly from traditional orchards of rather distant regions of Bosnia & Herzegovina and Croatia, including the sample from Austria. We concluded this SSR profile might be the basic one for 'Bistrica'. After several repetition of whole genotyping work we confirmed that the remaining 11 accessions have slightly different genotype to the basic one, mostly at a single locus (9 accessions) while two accessions had different alleles at two and three loci. Considering the complexity of plum hexaploid genome and number and gene diversity of applied SSR loci we assume these accessions have the same unizygote origin as previous 19 ones, and observed variation might be the result of spontaneous mutations.

## ACCURACY OF THE GENETIC ANALYSIS FOR EARLY SELECTION OF HAZELNUT (*Corylus avellana* L.) GENETIC RESOURCES IS INCREASED BY *in vitro* MICROPROPAGATION OF ZYGOTIC EMBRYOS

**P. Gutiérrez Pesce, G. Catarcione, E. Rugini\*, and C. De Pace**

Dept. of Science and Technologies for Agriculture, Forestry, Nature, and Energy,  
University of Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy  
e-mail: rugini@unitus.it

Early screening among seedling genotypes from full-sib (FS) progenies of the highly heterozygous fruit trees, such as hazelnut (*Corylus avellana* L.), requires accuracy in the analysis of the genetic variance compared to the variance due to the environmental effects. However, for developing a suitable statistical analysis the unique genotype obtained from each seedling needs to be cloned before carrying out multiple measurements in replicated trials and estimate the upper limit to the degree of genetic determination for a trait phenotype. We provide a method for seedling cloning through axillary shoot multiplication from the leaf nodes (micro-cuttings) of the primary shoot developed from zygotic embryos germinated *in vitro*. Embryos were obtained from FS seeds after controlled pollination among four clonal hazelnut landraces. About 50% of the excised embryos germinated *in vitro* and gave rooted seedlings with 5 - 7 leaf nodes. The proportion of micro-cuttings which proliferated an axillary shoot after excision from the primary embryo shoot at leaf node position "1" (the node right below the apical meristem of the seedling), "2" and "3", was  $\geq 0.9$ , but this proportion decreased to 0.6 for micro-cuttings taken from leaf node at position "4", and to 0.32 for the micro-cuttings from the basal node. These proportions were not significantly affected by the variation among FS seedlings from different maternal parent. The average number of axillary shoots originated from each seedling was 2.5. The germination medium composed of Cheng's mineral salts and supplemented with  $1.5 \text{ mg L}^{-1}$  Indol Butyric Acid (G-medium-C ) allowed root formation in  $\geq 80\%$  of the axillary shoots. Using the proposed protocol, the 4 - 6 ramets, needed for repeated measurements in the genetic analysis of hazelnut FS seedling trait, can be produced in less than three months.

## APPLICATION OF CHLOROPLAST DNA IN DETERMINATION OF *Punica granatum* PHYLOGENY

**B.E. Sayed Tabatabaei\* and Ebrahim Keyvanifar**

College of Agriculture, Isfahan University of Technology, Isfahan, Iran  
e-mail: sayedt@cc.iut.ac.ir

Pomegranate (*Punica granatum*) is an important horticultural and economical plant worldwide. Iran is one of the best known producers of pomegranate. In addition to its nutritional value for usual consumption, pomegranate also contains many compounds with anticancer, antioxidant and antimicrobial activities. Parts of the biosynthesis pathways of these compounds are believed to be located inside the plastids and therefore, study of the chloroplast genes may help with better understanding of the production mechanisms of these compounds. Pomegranate cpDNA from leaves of cultivated, wild and ornamental pomegranates were extracted and genes of interest were isolated using PCR reactions and specific primers designed based on aligned cpDNA sequences from other plants. PCR products were sequenced and have been used to construct three datasets. In summary it was found that sizes of the *rpl36* and *rpoA* genes were 114 and 1014bp, encoding 37 and 337 amino acids, respectively. Pomegranate *infA* gene comprised of 240bp that included 8 stop codons. Hence *infA* may be in fact a pseudogene. On the other hand bioinformatics analysis of the sequence 5' UTR pointed to the likely presence of promoters and therefore possible existence of a RNA editing process that modifies the gene internal stop codons to amino acid codons. Among cultivated, wild and ornamental pomegranates, *infA* gene was completely conserved while *rpl36* and *rpoA* genes showed few differences in their sequences. Based on the results of the total dataset, *Oenothera* was the closest evolutionary lineage to *Punica*. Coding regions and *rpoA* data sets grouped *Eucalyptus*, closer to *Punica* than *Oenothera*. Results from the nucleotide dataset of *rpoA* sequence were more congruent with recent studies of angiosperms phylogeny than those from other datasets.

## GENETIC DIVERSITY OF IRANIAN POMEGRANATE (*Punica granatum* L.) CULTIVARS USING MICROSATELLITE MARKERS

**B. Sharifnabi\*, S. Ebrahimi, and B.E. Sayed Tabatabaei**

Dept. of Agricultural Biotechnology, College of Agriculture, Isfahan University of Technology, Isfahan 8415683111, Iran

Pomegranate (*Punicagranatum* L.) is an important horticultural crop in semitropical regions of the world cultivation of pomegranate, as income generating fruit to the farmers and export commodity. Because of widely distribution of native germplasms across the country, pomegranate has enormous genetic variation for different agronomic and quality traits. Given the importance of this diversity, it is important to develop and utilize tools to evaluate genetic diversity among local genotypes to promote conservation and sustainable utilization of this crop. In this study, microsatellite markers were chosen for evaluation of genetic variation among local populations of pomegranate. To design microsatellite primers, two enriched genomic libraries with dinucleotide AG and trinucleotide ATG microsatellite motifs were constructed using magnetic beads. Positives clones have been detected by a simple PCR method contained SSR motifs. A total of 25 SSR primer pairs were designed from flanking regions of the repeats of these positive sequences and their efficiency was assessed among 20 local pomegranate germplasms including 11 commercial cultivars, seven wild and two ornamental genotypes. All primers amplified microsatellite loci in the selected genotypes; 12 of them showed only monomorphic bands, 11 primers amplified 13 polymorphic loci and two primer pairs amplified extra bands. Phylogenetic consensus tree was plotted. According to the result of these analyses, the mean number of alleles per polymorphic locus was 3.38 and mean of polymorphic information content (PIC) was calculated as 0.433. Five commercial genotypes were considered as an individual cluster and 15 genotypes were grouped into three different clusters. In this classification, clustering of the genotypes was in accordance of their geographical distribution; in addition, wild and domestic genotypes were divided in different clusters. The results demonstrated that magnetic bead enrichment method is a more efficient technique for identification of microsatellite motifs to design SSR markers for evaluating pomegranate genotypes.

## **EVALUATION OF GENETIC VARIABILITY INSIDE AN ITALIAN APPLE GERMPLASM COLLECTION**

**L. Dondini, P. De Franceschi, W. Liang, R. Paris, F. Gennari, S. Sansavini, and S. Tartarini\***

Dipartimento Colture Arboree Università di Bologna

The primary difficulties in apple breeding are the long juvenile period of seed-derived plants and the high degree of cultivar heterozygosity, a fact that is also dictated by the marked gametophytic self-incompatibility of this species. A panel of apple SSRs was chosen to analyse the genetic diversity among more than 400 apple accessions of Italian germplasm housed at the DCA-BO experimental fields of the Bologna University. The consequent cluster analysis made it possible to solve practical doubts between synonymy and homonymy of genotypes with similar behaviour and similar morphological traits and, more important, to describe the relationships among the Italian apple germplasm varieties that could represent a marvellous source of genetic diversity in which discover advantageous allelic forms of functional genes to be used in future breeding programs. This analysis made also possible the identification various triploid genotypes. The same set of cultivars was also phenotyped for the fruit quality traits. This work was carried out within the framework of the 'Fruit Breedomics EU project' and represents a preliminary step in the definition of an Italian apple 'core collection'. The wide panel of selected genotypes will be used to improve the knowledge of genetic variability distribution by exploring the phenotypic and allelic diversity available in the apple germplasm and, in this way, to identify the genomic regions contributing to the genetic control of major horticultural traits through genome-wide association genetics.

## IDENTIFICATION OF APPLE SPORT MUTANTS OF 'ANTONOVKA' BY MOLECULAR METHODS

O.Yu. Urbanovich<sup>1\*</sup>, P.V. Kuzmitskaya<sup>1</sup>, Z.A. Kazlouskaya<sup>2</sup>, and N.A. Kartel<sup>1</sup>

<sup>1</sup>Institute of Genetics and Cytology, 27, Akademicheskaya str., 220072, Minsk, Belarus

<sup>2</sup>Institute of Fruit Growing, 2, Kovaleva str., 223013 Samokhvalovichy, Minsk region, Belarus

e-mail: O.Urbanovich@igc.bas-net.by

Apple is distinguished for its high intraspecific polymorphism of some genome sequences that allows developing effective methods for its DNA-identification, in particular on the basis of SSR markers. These methods enable significant distinction between species, cultivars, hybrids, but not between sport mutants. However, sport mutants differing from the initial cultivar in morphological traits were described for some apple cultivars including old Russian cultivar 'Antonovka'. The present investigation was carried out for studying genetic diversity of sport mutants derived from the cultivar 'Antonovka' and for possibility of their identification by molecular methods. Ten accessions of this cultivar differing in morphological traits were used in this study. They were collected from orchards located in various regions of Belarus. SSR analysis was applied using 20 markers, which were known to have a high level of polymorphism among apple cultivars. The results obtained have shown that only one of ten accessions differed in the allele composition at the loci studied that indicated on its cross-pollination origin. The other nine accessions did not differ in the allele composition for these 20 SSR loci that does not allow effective identification of apple 'Antonovka' sport mutants to be provided by this approach. No differences between these accessions were revealed by methods of ISSR analysis using 10 primers and RAPD analysis using 27 primers. The S-SAP method, using 33 combinations of primers, one of which is bordering the sequence of a retrotransposon, was applied for detailed genetic analysis of 'Antonovka' sport mutants. Individual polymorphic fragments between accessions were revealed by this approach. It is possible that variation in mobile genetic elements during vegetative reproduction could result in genetic and morphological difference of sport mutants of the cultivar 'Antonovka'.



## **A GENETIC IDENTIFICATION METHOD FOR RASPBERRY (*Rubus idaeus*) AND BLACKBERRY (*Rubus fruticosus*) CULTIVARS BY MICROSATELLITES (SSR)**

**C. Fritzmann<sup>1</sup>, G. Muster<sup>2</sup>, and H. Xuan<sup>1\*</sup>**

<sup>1</sup>Kompetenzzentrum Obstbau Bodensee (KOB), Schuhmacherhof 6, 88213 Ravensburg, Germany

<sup>2</sup>LVWO Weinsberg, Traubenplatz 5 74189 Weinsberg, Germany  
e-mail: haibo@kob-bavendorf.de

The raspberry (*Rubus idaeus*) and the blackberry species (*Rubus fruticosus*) are economically important fruits in Europe. The accurate characterization of *Rubus* germplasm using morphological traits is difficult, molecular markers provide more reliable tools for cultivar identification and genetic diversity assessment. 15 SSR primers chosen from Castillo et al. (2010) and Graham et al. (2004) were used for identifying 22 raspberry and 14 blackberry cultivars, on the one hand, to determine the polymorphism of these markers, on the other hand, to establish one routine method for cultivar identification at the Kompetenzzentrum Obstbau-Bodensee (KOB) research station. Total genomic DNA was extracted from the freeze-fried samples according to the modified method of Neumüller (2004) by a CTAB (cetyl trimethyl ammonium bromide)-based extraction. DNA was quantified by the 'SERVA Digital Imaging and Analysis System DIAS-I', and subsequently diluted for the analysis. The primers were labelled with Cy5 and Cy5.5. Microsatellite fragments were detected and analyzed by capillary electrophoresis using the Beckman CEQ 8000 (Beckman Coulter, Inc.) by comparison with internal size standards. 15 SSRs revealed different polymorphism. The polymorphism information content (PIC), frequencies of 15 SSR loci distributed on these totally 36 cultivars are described and discussed.

## IDENTIFYING QUINCE CULTIVARS BY MEANS OF APPLE- AND PEAR MICROSATELLITES

D. Spann<sup>1</sup>, M. Neumüller<sup>2</sup>, and H. Xuan<sup>1\*</sup>

<sup>1</sup>Kompetenzzentrum Obstbau-Bodensee (KOB), Schuhmacherhof 6, D-88213 Ravensburg,

<sup>2</sup>TUM, Duernast 2, 85354 Freising, Germany  
e-mail: haibo@kob-bavendorf.de

In recent years, the methods for fingerprinting the cultivars of apple, pear, cherry and European plum by means of microsatellites (SSR) were established at the Kompetenzzentrum Obstbau-Bodensee (KOB) research station, Ravensburg, South of Germany and a database is just in buildup. As a practical technique, to examine the authenticity and purity of cultivars for plant nurseries; for fruit import and export chains; and also to support the government quality inspection service in the area of food trade are expected. The quince (*Cydonia oblonga*) belongs to the family *Rosaceae*, subfamily *Maloideae* along with pears (*Pyrus* spp.) and apples (*Malus* spp.), is cultivated for over 4000 years and is one of the oldest types of fruit. The quince is closely related to apple and pears which is reflected in the same chromosome number of  $x = 17$  and  $2n = 2x = 34$ . In this work, identification of quince cultivars by means of apple- and pear microsatellites was carried out. Three to five uninfected young fresh leaves from each cultivar were collected, and immediately processed or freeze-dried and stored in the freezer at  $-32^{\circ}\text{C}$ . 15 apple and 2 pear SSRs were chosen from the international gene bank for the characterization of the quince cultivars. The extraction of genomic DNA succeeded by means of a mini plant kit from Qiagen. The PCR fragments were detected by an 8-capillary sequencer (CEQ 8000, Beckman & Coulter). The polymorphism information content (PIC), frequencies of 15 SSR are described and discussed.

## EVALUATION OF HONEYSUCKLE (*Lonicera* sp. L.) GERMPLASM COLLECTION USING DNA MARKERS

D. Naugzemys<sup>1,2</sup>, S. Zilinskaitė<sup>2\*</sup>, A. Skridaila<sup>2</sup>, V. Kleizaitė<sup>1</sup>, and D. Zvingila<sup>1</sup>

<sup>1</sup>Vilnius University, Department of Botany and Genetics, M.K. Ciurlionio 21/27, LT-03101, Vilnius, Lithuania

<sup>2</sup>Vilnius University Botanical Garden, Kairenu 43, LT-10239, Vilnius, Lithuania

Development of new cultivars largely depends on genetic collections containing genotypes distinguishing by valuable and accessible genetic diversity. In order to use a collection efficiently, estimation of its genetic diversity using molecular methods is necessary, because variability of morphological markers usually is insufficient for effective management of genetic resources. Multiple studies show that RAPD method is one of the most suitable for assessment of genetic diversity in genetically uncharacterized species: it does not require the prior knowledge of primer target sequence. We used RAPD method for genotyping accessions of an under-utilized fruit blue honeysuckle (*Lonicera caerulea* L.) collection of Vilnius University Botanical Garden. Accessions of other honeysuckle species were also included in this study. After initial screening of 50 primers an optimal set of twelve primers was determined for the evaluation of the collection. These primers were used to genotype a hundred accessions of different taxonomic rank and breeding value, including species, subspecies, cultivars and genetic lines. The estimation of DNA polymorphism in the groups of accessions representing species, subspecies, cultivars and genetic lines showed that the greatest polymorphism, as expected, was between species (97.8%). The level of DNA polymorphism in the group of accessions of *L. caerulea* was 83.2%. DNA polymorphism was sufficient to genotype all studied honeysuckle plants. One RAPD locus out of total set of 149 was specific to all samples attributed to complex polymorphic species *L. caerulea*. Species-specific RAPD marker can be used for the identification of *L. caerulea* and its hybrids in the breeding process.

## PRIMARYLY IDENTIFICATION OF PEAR VARIANTS AND ANALYSIS OF THEIR GENETIC RELATIONSHIP BY SRAP MARKERS

Z. Li-wu\*, X. Ao-jing, J. Bing, H. Wei, and Y. Zhen-feng

The Key Laboratory of Pomology, Anhui Agricultural University, Hefei, 230036

‘Cuiguan’ [‘Kousui’ × (‘Shinseiki’ × ‘Hangqing’)] is a main early cultivar from Asian pear (*Pyrus pyrifolia* Nakai) published in China. The two morphological variants were found in ‘Cuiguan’ pear orchard in 2002 and coded as ‘02-1’ and ‘02-2’, respectively. To identify if there is genetic variation happened in the two morphological variants, the genomic DNA of 27 selected accessions, including variant ‘02-1’ and ‘02-2’, were studied by method of sequence-related amplified polymorphism (SRAP) markers. After the SRAP-PCR reaction system appropriate for pear plant was optimized with orthogonal design, 22 pairs of suitable primers screened out from 88 ones were used to detect the different fragments in these pear cultivars. The genetic relationships between accessions were analyzed by method of hierarchical cluster analysis using the SPSS (Statistical Package for the Social Science) 10.0 software. The result showed that the optimal SRAP-PCR system for pear plant was composed of 2.4 mmol·L<sup>-1</sup> Mg<sup>2+</sup>, 0.1 mmol·L<sup>-1</sup> dNTPs, 1.5 U DNA polymerase, 80 ng DNA template, and 0.18 μmol·L<sup>-1</sup> primer in 25 μl total reaction volume. There were 196 fragments amplified in all tested accessions with the 22 pairs of suitable primers, the size of that was from 0.1 kb to 2.2 kb. Compared with the control cultivar ‘Qingxiang’ pear, seven differential fragments were detected in variant ‘02-1’ and thirteen in variant ‘02-2’. It was proved that there was genetic variation in the DNA level of the two morphological variants. The variant ‘02-1’ and ‘Qingxiang’ pear clustered at closer distance showed they had a nearer relationship, while the variant ‘02-2’ fell into a cluster with ‘Shinseiki’ and ‘Huanghua’ pear firstly, and then clustered with ‘Qingxiang’ pear. It was postulated that the variant ‘02-1’ was a mutant of ‘Qingxiang’ pear, and the variant ‘02-2’ may be a seedling strain and have the same parentage with ‘Qingxiang pear’.



## LIST OF PRESENTING AUTHORS

Surname		Page
Acar	I.	31
Ahmadi-Afzadi	M.	189
Babaei	F.	151, 215
Baciu	A.	93
Bahar	M.	40
Bastiaanse	H.	86
Belaj	A.	94, 133
Ben Sadok	I.	52
Bendokas	V.	190
Bester	C.	163
Bestfleisch	M.	95
Blažek	J.	152
Bokszczanin	K.L.	63
Brandi	F.	164
Bus	V.	80
Česonienė	L.	96, 134
Cheng	C.	61
Choraży	A.	191
Cipriani	G.	97
Dardeniz	A.	111
Datson	P.	74
Daubaras	R.	98, 135
De Wit	I.	112
Dekena	D.	153
Den Nijs	T.	67
Dumitru	L.M.	113
Elbakkali	A.	41
Ershadi	A.	154
Evans	K.M.	33, 165
Fahrentrapp	J.	82
Farsi	M.	216
Feldmane	D.	114
Fernández-Fernández	F.	83
Garkava-Gustavsson	L.	192
Gelvonauskiene	D.	193
Gelvonauskis	B.	136
Gharaghani	A.	137
Girichev	V.S.	155
Grāvīte	I.	115
Guo	G.	99
Hampson	C.R.	116, 194
Hanke	M.-V.	–
Hāršan	E.	117
Human	J.P.	62
Ikase	L.	32
Imani	A.	100

Surname		Page
Indreias	A.	118
Ion	L.	195, 196, 197, 198
Irisarri	P.	166
Isuzugawa	K.	167
Jajarmi	V.	156
Jiang	S.	157
Kazlouskaya	Z.A.	199, 200
Kellerhals	M.	79
Khorramdelazad	M.	138, 217
Kim	D.	36, 139, 140, 168
Király	I.	218
Kolahi Zenoozi	S.	179
Korbin	M.	201
Kovács	S.	141
Krupa	T.	169
Kumar	S.	72
Lācis	G.	219
Lateur	M.	101, 170
Laurens	F.	71
Le Roux	P.-M.	69, 202
Leida	C.	158
Leone	A.	220
León	P.J.	171
Lewandowski	M.	120
Li	X.	102
Li-wu	Z.	236
Lukoseviciute	V.	221
Luro	F.	45, 60
Majidian	P.	222, 223
Miller	D.D.	103
Mirzwa-Mróz	E.	203
Moriya	S.	204
Mousavi Derazmahalleh	S.M.	224
Nam	E.Y.	142
Nikoumanesh	K.	225
Noh	J.H.	119
Nybom	H.	226
Olbricht	K.	104, 143
Paduch-Cichal	E.	205
Papstein	F.	121, 144
Patocchi	A.	84
Pejić	I.	227
Perchepied	L.	206
Piesterzeniewicz	C.	122

<b>Surname</b>		<b>Page</b>
Pieterse	W-M.	73
Pina	A.	53, 145
Piripireivatlou	S.	159
Pitera	E.	105
Przybyła	A.A.	70
Reighard	G.	207
Revers	L.F.	34
Rubio-Cabetas	M.J.	87, 180
Rugini	E.	106, 228
Sansavini	S.	29
Sargent	D.J.	75
Savelyev	N.I.	58
Sayed Tabatabaei	B.E.	229
Schuster	M.	35, 123, 124
Seker	M.	146, 172
Shan	F.	30
Sharifnabi	B.	230
Sikorskaite	S.	208
Socias i Company	R.	46, 47, 147, 173
Socquet-Juglard	D.	209
Szpadzik	E.	125
Tantasawat	P.	210
Tartarini	S.	181, 231
Terakami	S.	182
Testolin	R.	39
Tomala	K.	129
Tóth	M.	126
Univer	T.	127
Urbanovich	O.Yu.	232
Vogt	J.	59
Volz	R.K.	57
Wang	Z.	128, 174
Wei	H.	175
Wenzel	S.	183
Wińska-Krysiak	M.	176
Wöhner	Th.	85
Wrona	D.	130
Würdig	J.	68
Xuan	H.	233, 234
Yamamoto	T.	184
Yun	H.K.	185
Zilinskaitė	S.	235
Zini	E.	211
Zuriaga	E.	81, 186
Żurawicz	E.	51

