Slide 17

Plastidial DNA barcoding for assessing gene pool diversity in common bean (*Phaseolus* spp.)

✔ **DNA barcoding: case study #1**

Plant materials: landraces belonging to Mesoamerican and Andean gene pools

- **18 Central America**
  Mexico (13)
  Guatemala (1)
  Costa Rica (1)
  El Salvador (1)
  Honduras (1)
  Venezuela (1)

- **12 Southern America**
  Argentina (6)
  Bolivia (2)
  Peru (1)
  Cile (1)
  Colombia (1)
  Equador (1)

PLANT MATERIALS
List of 30 common bean landraces from the American gene pools

ISBN 0-9670694-0-8

Gianni Barcaccia
Padua University

Orto Botanico di Padova – 3 Maggio 2011   17

Slide 18

Plastidial DNA barcoding for assessing gene pool diversity in common bean (*Phaseolus* spp.)

✔ **DNA barcoding: case study #1**

Plant materials:
- 25 Italian varieties

- Accessions of *P. coccineus*, *P. lunatus* and *Vigna unguiculata* were adopted as out-types.

1. Cannellino rosso (Pv)
2. Riso giallo (Pv)
3. Montalbano (Pv)
4. Munachedda nera (Pv)
5. San Michele (Pv)
6. Nasieddu viola (Pv)
7. Maruchetta (Pv)
8. Riso bianco (Pv)
9. Cannellino nano (Pv)
10. Verdolino (Pv)
11. Blu lake (Pv)
12. Goldrush (Pv)
13. Clio (Pv)
14. BAT881 (Pv)
15. Lena (Pv)
16. Giulia (Pv)
17. Saluggia nano (Pv)
18. Venere (Pv)
19. Borlotto Lamon (Pv)
20. Saluggia (Pv)
21. Cannellino (Pv)
22. Verdone (Pv)
23. San Matteo (Pv)
24. Zolferino rovigotto (Pv)
25. Nero messicano (Pv)
26. F. di Lima (Pl)
27. F. di Spagna (Pc)
28. F. dall'occhio (Vu)

PLANT MATERIALS
List of 25 Italian common bean varieties

ISBN 0-9670694-0-8

Gianni Barcaccia
Padua University

Orto Botanico di Padova – 3 Maggio 2011   18

Slide 19

Plastidial DNA barcoding for assessing gene pool diversity in common bean (*Phaseolus* spp.)

✔ **Primers for bean DNA barcoding**

Design of primers to be used as chloroplast and nuclear barcodes.

Forward ——→   SNPs + In/Dels   ←—— Reverse

TOOLS AND METHODS
How to design forward and reverse primers

ISBN 0-9670694-0-8

Gianni Barcaccia
Padua University

Orto Botanico di Padova – 3 Maggio 2011   19

Slide 20

Plastidial DNA barcoding for assessing gene pool diversity in common bean (*Phaseolus* spp.)

✔ **Primers for bean DNA barcoding**

List of primers used for chloroplast and nuclear markers.

Marker	Amplicon length (bp)				Primer name	Primer sequence (5'-3')
	P. vulgaris	*P. coccineus*	*P. lunatus*	*V. uguiculata*		
rbcL gene	543	543	543	543	rbcL_F	GCAGCATTYCGAGTAASTCCYCA
					rbcL_R	GAAACGYTCTCTCCAWCGCATAAA
					rbcL 724R*	TCACATGTACCTGCAGTAGC
matK gene	695	695	695	695	matK4La	CCTTCGATACTGGGTGAAAGAT
					matK1932Ra	CCAGACCGGCTTACTAATGGG
trnL intron	350	350	296	357	trnL_F	GGATAGGTGCAGAGACTCRATGGAAG
					trnL_R	TGACATGTAGAATGGGACTCTATCTTTAT
					5'trnLUAAF*	CGAAATCGGTAGACGCTACG
					3'trnLUAAR*	GGGGATAGAGGGACTTGAAC
atpB-rbcL IGS	329	325	326	331	atpB_F	GGTACTATTCAATCAATCCTCTTTAATTGT
					atpB_R	ATGTAAATCCTAGATGTRAAAATAKGCAG
					atpB_R2*	CGCAACCCAATCTTTGTTTC
trnH-psbA IGS	365	365	365	369	psbA3'I	GTTATGCATGAACGTAATGCTC
					trnHf	CGCATGGTGGATTCACAATCC
rpoB-trnC IGS	1117	1117	1124	1136	rpoB_F	CKACAAAAYCCYTCRAATTG
					trnCGCAR	CACCCRGATTYGAACTGGGG
					rpoB_R3*	TTCTTTACAATCCCGAATGG
trnT-trnL IGS	813	837	823	871	trnTUGU2F	CAAATGCGATGCTCTAACCT
					5'trnLUAAR	TCTACCGATTTCGCCATATC
Total length	3556	3576	3509	3627		
ITS1	373	382	355-364	314	ITS5	GGAAGTAAAAGTCGTAACAAGG
					ITS2	GCTGCGTTCTTCATCGATGC
ITS2	419	418	413	401	ITS3	GCATCGATGAAGAACGCAGC
					ITS4	TCCTCCGCTTATTGATATGC

TOOLS AND METHODS
Plastid and nuclear targets for DNA barcoding in common bean

ISBN 0-9670694-0-8

Gianni Barcaccia
Padua University

Orto Botanico di Padova – 3 Maggio 2011   20

Slide 21

Plastidial DNA barcoding for assessing gene pool diversity in common bean (*Phaseolus* spp.)

✔ **Bean DNA barcoding: experimental strategy**

1. Amplification by sequence-tagged site PCR

2. Sequencing of DNA amplicons

3. Multi-alignment of DNA sequences

STRATEGY
Experimental methods for DNA barcoding in common bean

ISBN 0-9670694-0-8

Gianni Barcaccia
Padua University

Orto Botanico di Padova – 3 Maggio 2011   21

Slide 22

Plastidial DNA barcoding for assessing gene pool diversity in common bean (*Phaseolus* spp.)

✔ **Bean DNA barcoding: results**

Basic information on the cpDNA and ITS barcode regions, including sequence length of amplicons, inter- and intra-specific number and frequency of SNPs and IN/DELs.

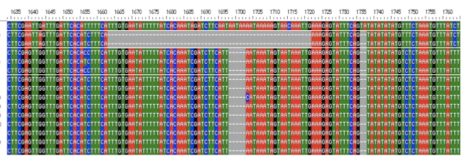|	rbcL	matK	trnL	atpB-rbcL	trnH-psbA	trnT-trnL	rpoB-trnC	ITS1	ITS2
Total No. of *Phaseolus* entries	63	63	63	63	63	63	63	63	63
Average amplicon length (bp)	543	695	338	328	366	836	1124	358	413
No. SNPs in *Phaseolus* spp.	8	n.d.	21	14	14	53	48	65	58
Interspecific frequency (SNPs/100 bp)	1.5	n.d.	6.0	4.3	3.8	6.5	4.2	17.4	13.8
No. SNPs in *P. vulgaris*	0	n.d.	4	0	8	3	2	6	4
Intraspecific frequency (SNPs/100 bp)	0	n.d.	1.1	0	2.2	0.4	0.2	1.6	1.0
No. of In/Dels in *Phaseolus* spp.	0	n.d.	1	4	0	5	5	10	5
Average In/Del size (bp)	0	n.d.	58	2	0	7	2	4	5
No. of heterozygous sites	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	3	7
Amplification success (%)	100%	100%	100%	100%	100%	100%	100%	100%	100%
Sequencing success (%)	100%	62%	100%	100%	100%	100%	90%	97%	100%

- The average frequency was 0.8 SNPs/100 bp (cpDNA) and 1.3 SNPs/100 bp (ITS) at the intraspecific level (*P. vulgaris*).

RESULTS
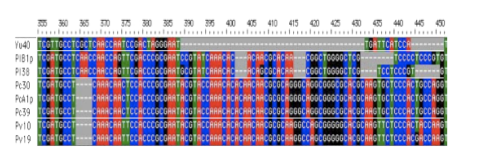Main statistics from cpDNA and ITS barcodes

ISBN 0-9670694-0-8

Gianni Barcaccia
Padua University

Orto Botanico di Padova – 3 Maggio 2011   22

Slide 23

Plastidial DNA barcoding for assessing gene pool diversity in common bean (*Phaseolus* spp.)

✔ **Bean DNA barcoding: results**

Polymorphism information content of the investigated plastid genic regions

RESULTS

Finding SNPs and IN/DELs between *Vigna unguiculata* and *Phaseolus* species

ISBN 0-9670694-0-8

- Several SNPs and IN/DELs were found between *Phaseolus* species and *Vigna unguiculata*

Gianni Barcaccia
Padua University

Orto Botanico di Padova – 3 Maggio 2011   23

Slide 24

Plastidial DNA barcoding for assessing gene pool diversity in common bean (*Phaseolus* spp.)

✔ **Bean DNA barcoding: results**

IN/DELs and SNPs among *Phaseolus* spp. varieties

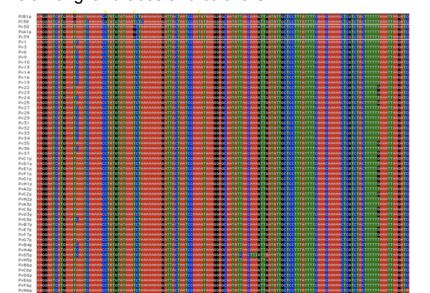**RESULTS**

Finding SNPs and IN/DELs among *Phaseolus* species

ISBN 0-9670694-0-8

Gianni Barcaccia
Padua University

Orto Botanico di Padova – 3 Maggio 2011   24

Slide 25

Plastidial DNA barcoding for assessing gene pool diversity in common bean (*Phaseolus* spp.)

✔ **Bean DNA barcoding: results**

SNPs among landraces and cultivars
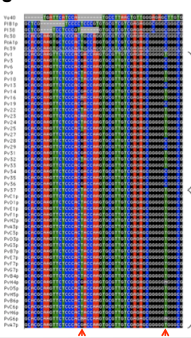
RESULTS

Finding SNPs within *Phaseolus* species

ISBN 0-9670694-0-8

Gianni Barcaccia
Padua University

Orto Botanico di Padova – 3 Maggio 2011   25

Slide 26

Plastidial DNA barcoding for assessing gene pool diversity in common bean (*Phaseolus* spp.)

✔ **Bean DNA barcoding: results**

SNPs among landraces and cultivars

RESULTS

Finding SNPs between *Phaseolus* gene pools and among landraces

ISBN 0-9670694-0-8

Italian cultivars

Mesoamerican and Andean landraces

Gianni Barcaccia
Padua University

Orto Botanico di Padova – 3 Maggio 2011   26

Slide 27

Plastidial DNA barcoding for assessing gene pool diversity in common bean (*Phaseolus* spp.)
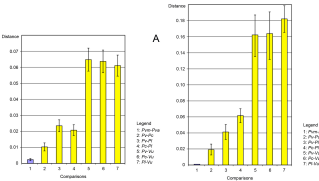
✔ **Bean DNA barcoding: results**

Mean and standard deviation of inter- and intra-specific genetic divergences calculated using the K2P distance model for the cpDNA markers and ITS regions, and overall.

Interspecific K2P distance	rbcL	trnL	atpB-rbcL	trnH-psbA	trnT-trnL	rpoB-trnC	Overall	St. Dev.	ITS1	ITS2	Overall	St. Dev.
P. vulgaris/*P. coccineus*	0.0037	0.0139	0.0072	0.0107	0.0088	0.0070	0.01035	0.0250	0.0105	0.0169	0.0173	0.0065
P. vulgaris/*P. lunatus*	0.0074	0.0250	0.0204	0.0226	0.0227	0.0209	0.02314	0.0389	0.0650	0.0438	0.0432	0.0107
P. vulgaris/*Vigna unguiculata*	0.0168	0.0459	0.0515	0.0382	0.0852	0.0571	0.06181	0.00718	0.2617	0.1671	0.1651	0.0231
Intraspecific K2P distance (*P. vulgaris*)	0.0000	0.0041	0.0001	0.0030	0.0008	0.0006	0.00213	0.0066	0.0002	0.0016	0.0006	0.0003
St. Dev.	0.0000	0.0023	0.0001	0.0015	0.0005	0.0004	0.00069		0.0002	0.0005	0.0003	

RESULTS

The genetic divergence between *Phaseolus vulgaris* accessions was very low!

ISBN 0-9670694-0-8

Genetic distances within and between *Phaseolus* spp. and Vigna:

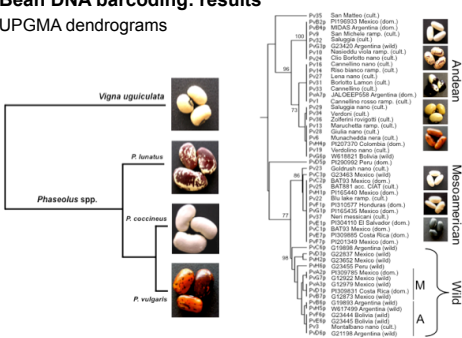inter- and intra-specific genetic distances referred to cpDNA markers (A) and ITS regions (B).

A

B

Gianni Barcaccia
Padua University

Orto Botanico di Padova – 3 Maggio 2011   27

Slide 28

Plastidial DNA barcoding for assessing gene pool diversity in common bean (*Phaseolus* spp.)

✔ **Bean DNA barcoding: results**

UPGMA dendrograms

RESULTS

DNA barcoding is useful to identify *Phaseolus* species and to resolve diversity between bean species

ISBN 0-9670694-0-8

Vigna uguiculata

Phaseolus spp.

P. lunatus

P. coccineus

P. vulgaris

Andean

Mesoamerican

Wild

Gianni Barcaccia
Padua University

Orto Botanico di Padova – 3 Maggio 2011   28

Slide 29

Plastidial DNA barcoding for assessing gene pool diversity in common bean (*Phaseolus* spp.)

✔ **Bean DNA barcoding: results**

Consensus sequence related to the 17 individual SNPs detected in the target cpDNA regions with information on the haplotypes found across all entries.

Table 4: Consensus sequence related to the 17 individual SNPs detected in the target cpDNA regions with information on the haplotypes found across all common bean (*P. vularis*) entries. Haplotypes are arranged in three main subgroups for ancestrals, mesoamerican and andean gene pools.

Marker		trnL intron				trnH-psbA									trnT-trnL		rpoB-trnC	
SNP position	14	183	264	332	156	219	223	224	225	229	272	283		85	512	673	478	642
Consensus sequence	G	A	T	T	A	T	A	A	A	G	T	C		A	A	T	G	A
Haplotype	No. Entries																	
Hap16	2	A		A			C	T	T	T	A							
Hap09	1					C	C	T	T	T	A							n.d.
Hap01	1		C															
Hap03	10			G									G			G		C
Hap08	1			G									G			G		C
Hap12	1			G									G					
Hap13	3	A		G	A								G			G		C
Hap06	7	A			A	C												
Hap14	1	A	C		A													
Hap15	3	A			A													
Hap02	15					C												
Hap04	3														C			T
Hap10	1			G											C	G		T
Hap11	1			G											C			T
Hap07	1													A				n.d.
Hap05	6																	

n.d., not determined.

Hap01: PvA2md
Hap02: PvA7ad, PvG8aw, PvG3aw, Pv84ad, Pv1tc, Pv6itc, Pv9itc, Pv10tc, Pv13itc, Pv14itc, Pv16tc, Pv19itc, Pv39tc, Pv54tc
Hap03: PvC3mw, PvG1md, PvC1ad, Pv41md, PvC2ad, PvE7md, PvH8ad, PvF1md, Pv22tc, Pv23tc
Hap04: PvH8aw, PvD6aw, Pv3ltc
Hap05: PvH3mw, Pv43mw, PvB7mw, PvE6aw, PvF6aw, PvD1md
Hap06: PvH4md, Pv28itc, Pv29itc, Pv31itc, Pv33itc, Pv34itc, Pv36itc
Hap07: PvH8aw
Hap08: PvD3mw
Hap09: PvD5ad
Hap10: PvB6aw
Hap11: PvE1md
Hap12: PvE1md
Hap13: PvF7md, Pv35tc, Pv37tc
Hap14: PvG7mw
Hap15: Pv88mw, PvC8mw, PvD8aw
Hap16: PvF8wanc, PvG8wanc

RESULTS
SNP-based haplotypes may discriminate Andean and Mesoamerican gene pools

Legend:
Ancestrals (yellow)
Mesoamerican gene pool (orange)
Andean gene pool (red)

Orto Botanico di Padova – 3 Maggio 2011 29

Gianni Barcaccia
Padua University

Slide 30

Plastidial DNA barcoding for assessing gene pool diversity in common bean (*Phaseolus* spp.)

✔ **Bean DNA barcoding: results**

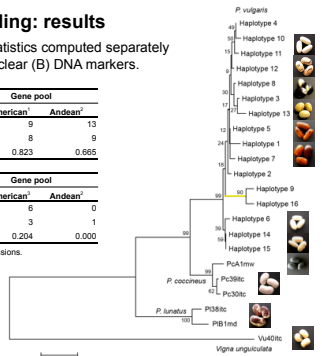Summary of haplotype statistics computed separately for chloroplast (A) and nuclear (B) DNA markers.

A

Genetic diversity statistics	Gene pool	
	Mesoamerican[1]	Andean[2]
No. segregating sites (S)	9	13
Haplotype number (Hn)	9	8
Haplotype diversity (Hd)	0.823	0.665

B

Genetic diversity statistics	Gene pool	
	Mesoamerican[3]	Andean[2]
No. segregating sites (S)	6	0
Haplotype number (Hn)	3	1
Haplotype diversity (Hd)	0.204	0.000

[1]29 accessions; [2]26 accessions; [3]28 accessions.

NJ tree based on the haplotypes identified for *Phaseolus* accessions.

RESULTS
Genetic diversity is about 25% higher in the Mesoamerican than in the Andean gene pool

Orto Botanico di Padova – 3 Maggio 2011 30

Gianni Barcaccia
Padua University

Slide 31

Plastidial DNA barcoding for assessing gene pool diversity in common bean (*Phaseolus* spp.)

✔ **Bean DNA barcoding: results**

Population structure of *Phaseolus vulgaris* germplasm core collection as estimated with STRUCTURE software

RESULTS
The population structure analyses revealed 3 distinct subgroups
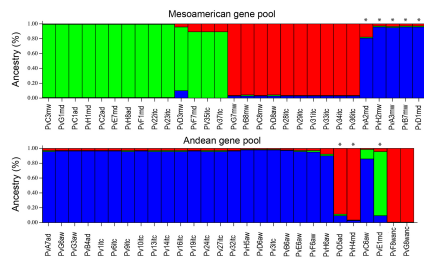
- Each accession is represented by a vertical histogram portioned into K=3 colored segments that represent the estimated membership of each individual.

Orto Botanico di Padova – 3 Maggio 2011 31

Gianni Barcaccia
Padua University

Slide 32

Plastidial DNA barcoding for assessing gene pool diversity in common bean (*Phaseolus* spp.)

✔ **Bean DNA barcoding: results**

Genetic diversity and differentiation of *P. vulgaris* accessions

RESULTS
Most Italian varieties (*i.e.* pure lines) are genetically differentiated from American landraces
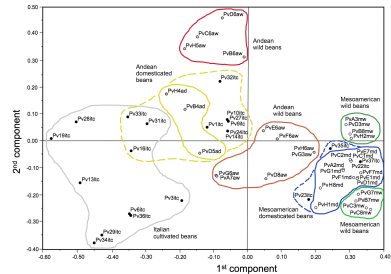
- Italian varieties originated from both Andean and Mesoamerican gene pools.

Orto Botanico di Padova – 3 Maggio 2011 32

Gianni Barcaccia
Padua University

Slide 33

Plastidial DNA barcoding for assessing gene pool diversity in common bean (*Phaseolus* spp.)

✔ **Bean DNA barcoding: concluding remarks**

- Strategy: multi-locus vs. single-locus barcoding
  - The occurrence of SNPs was lower in cpDNA barcodes than ITS1-2 regions (0.8 vs. 1.3 per 100 bp);
- Strategy: NJ tree-based vs. Haplotype-based
  - The NJ method is a powerful approach for estimating genetic differentiation among species, but it is relatively uninformative for studying genetic diversity within species;
  - The character-based method is able to identify several distinct haplotypes over all target regions corresponding to Mesoamerican or Andean accessions.
- Most domesticated Andean varieties were clustered apart from Mesoamerican varieties;
  - The Italian varieties originated from both Andean (15) and Mesoamerican (10) gene pools.

CASE STUDY
Concluding remarks on DNA barcoding in common beans

Orto Botanico di Padova – 3 Maggio 2011 33

Gianni Barcaccia
Padua University

Slide 34

Plastidial DNA barcoding for assessing gene pool diversity in common bean (*Phaseolus* spp.)

✔ **Bean DNA barcoding: concluding remarks**

- The DNA barcoding based on plastid genic and intergenic sequences confirmed to be a very powerful tool for reconstructing genetic relationships among plant species.

- Our findings raise some concerns about the use of plastid DNA barcoding for intraspecific genetic diversity studies in plant species and highlights its limitations for resolving genetic relationships between landraces and cultivars.

CASE STUDY
Use of cpDNA barcoding in common bean

Orto Botanico di Padova – 3 Maggio 2011 34

Gianni Barcaccia
Padua University

Slide 35

Case study #2: Nuclear DNA barcoding for assessing genetic identity of grapevine cultivars

Università degli Studi di Padova

BIODIVERSITÀ — IERI OGGI DOMANI

✔ **Identification of grapevine cultivars**

DNA barcoding as a tool for testing genetic identity within species!

BACKGROUND
Identification of grapevine cultivars (*i.e.* below the species level)

ISBN 0-9670694-0-6

Ampelography
→ leaves, shoots, flowers, bunches, berries, etc.
Protein/Metabolite profiling
→ proteins, secondary metabolites (anthocyanins)
DNA genotyping
→ SSR markers (Vitis Intl. Catalogue of Cultivars)

Gianni Barcaccia
Padua University

Orto Botanico di Padova – 3 Maggio 2011   35

Slide 36

Nuclear DNA barcoding for assessing the genetic identity of grapevine cultivars

Università degli Studi di Padova

BIODIVERSITÀ — IERI OGGI DOMANI

✔ **Multilocus DNA barcoding of grapevine germplasm**

• Implementation of a molecular diagnostic assay for the genetic identification of grapevine cultivars, grapes and wines through the reconstruction of cultivar-specific haplotypes;

GOALS
Multilocus DNA barcoding in *Vitis vinifera* L.

ISBN 0-9670694-0-6

• Resolution of homonymy/synonymy of grapevine cultivars;
• Traceability of grapevine-derived products (grapes and wines).

Gianni Barcaccia
Padua University

Orto Botanico di Padova – 3 Maggio 2011   36

Slide 37

Nuclear DNA barcoding for assessing the genetic identity of grapevine cultivars

Università degli Studi di Padova

BIODIVERSITÀ — IERI OGGI DOMANI

✔ **Plant materials: *Vitis* spp. accessions**

A total of 152 accessions of *Vitis vinifera* L. were analyzed for the discovery of single-copy gene-specific SNPs

PLANT MATERIALS
152 accessions of *Vitis vinifera* L. and 5 wild species as outgroups

ISBN 0-9670694-0-6

V. labrusca
V. riparia
V. berlandieri
V. rupestris
V. cinerea

V. vinifera L.

• 5 outgroups
• 127 European certified cultivars
• 25 Italian local cultivars

Gianni Barcaccia
Padua University

Orto Botanico di Padova – 3 Maggio 2011   37

Slide 38

Nuclear DNA barcoding for assessing the genetic identity of grapevine cultivars

Università degli Studi di Padova

BIODIVERSITÀ — IERI OGGI DOMANI

✔ **Selection of barcode regions**

Common target genes for DNA barcoding

Plastidial genes and intergenic regions
CBOL Plant Working Group

rbcL
matK
rpoB
rps16
Rpl32i

trnH-psbA
trnT-trnL
atpB-rbcL
psbK-psbI

Gene[a]	Genomic location	Number of sequences			
		Animals	Plants	Protists	Fungi
COI-barcode[b]	Mitochondria	195 777	520	1 931	410
16S-rDNA	Mitochondria	41 381	221	2 059	285
cytb	Mitochondria	88 324	165	1 920	1 084
ITS1-rDNA	Nucleus	12 175	57 693	68 839	56 675
ITS2-rDNA	Nucleus	13 923	58 065	67 332	56 349
18S-rDNA	Nucleus	21 063	17 121	32 290	33 327
rbcL	Plastid	NA[c]	30 663	37 328	NA[c]

[a]Gene abbreviations: COI, cytochrome c oxidase I; cytb, cytochrome bc1; ITS, internal transcribed spacer; rbcL, large subunit of ribulose 1,5-bisphosphate carboxylase/oxygenase.
[b]COI-barcode statistics are retrieved from Barcode of Life Data systems (http://www.barcodinglife.org).
[c]NA, not applicable.

TOOLS AND METHODS

Selection of the target sequences for DNA barcoding

ISBN 0-9670694-0-6

Single-copy genes of the nuclear DNA
List of primers for each nuclear gene along with basic information

Marker	Chromosome	Gene product	Length (bp)	Primer name	Primer sequence (5'-3')	Ta (°C)	References
GAI[a]	1	Transcription factor for gibberellins	761	GAI_F	ATGGATGAGCTTCTCGGCTGT	50	Wen et al. (2007)
				GAI_R	TAGAAGTGCATCTGRAGAAT		
ID04[b]	3	Predicted ZIP DNA-binding protein	419	ID04_F	CACCAGTCCCTTACCAGTCT	55	Salmaso et al. (2004)
				ID04_R	CAGTAGAGGAACACAACTGAG		
IIC08[c]	3	Predicted zinc-finger protein	418	IIC08_F	CAAGGCCTTCTCTTCGGTACC	60	Salmaso et al. (2004)
				IIC08_R	AAGAATTCATATCGCCGACC		
ATPS	7	ATP synthase	800	ATP_F	ATGCTGTTCCAGTCCGTTTC	60	Nicolè et al. (unpublished)
				ATP_R	GGGTCGATGGTGATCTTCT		
UFGT[d]	16	UDP-glucose:flavonoid 3-O glucosyltransferase	919	UFGT_F4	ATGTCTCAAACCACCACCAACC	63	Nicolè et al. (unpublished)
				UFGT_R3	TGACGGTGCCAAAGCTAATG		

[a]Biosynthetic pathway of gibberellins; [b]Transcription regulator and [c]Defence-related proteins from the Vitis EST database; [d]Biosynthesis of anthocyanins.

Gianni Barcaccia
Padua University

Orto Botanico di Padova – 3 Maggio 2011   38

Slide 39

Nuclear DNA barcoding for assessing the genetic identity of grapevine cultivars

Università degli Studi di Padova

BIODIVERSITÀ — IERI OGGI DOMANI

✔ **Strategy adopted for SNP discovery and CV haplotyping**

Gene (full-length)
5' UTR   3' UTR

Multiple alignments

Forward1   Forward2   Reverse1   Reverse2

Consensus

Amplicons

STRATEGY
Experimental strategy adopted for DNA barcoding

ISBN 0-9670694-0-6

PCR   Subcloning   Sequencing   SNP discovery

Haplotyping

Gianni Barcaccia
Padua University

Orto Botanico di Padova – 3 Maggio 2011   39

Slide 40

Nuclear DNA barcoding for assessing the genetic identity of grapevine cultivars

Università degli Studi di Padova

BIODIVERSITÀ — IERI OGGI DOMANI

✔ **Discovery of discriminant SNPs**

SNPs among *Vitis* species

V. riparia
V. rupestris
V. riparia
V. cinerea
V. labrusca
V. vinifera

SNPs among *V. vinifera* cultivars: 1 SNP/34 bp (23-51)

535 V. vinifera
534 V. labrusca
533 V. cinerea
532 V. berlandieri
530 V. riparia
531 V. rupestris

SNPs among biotipes within *V. vinifera* cultivars

Pinot bianco
Pinot grigio
Pinot nero
Pinot nero

Moscato bianco
Moscato sardo
Moscato giallo
Moscato amburgo

RESULTS
Discovery of discriminant SNPs either between *Vitis* species or within *V. vinifera* species

ISBN 0-9670694-0-6

Heterozygous SNPs in *V. vinifera* cultivars
UFGT

Gianni Barcaccia
Padua University

Orto Botanico di Padova – 3 Maggio 2011   40

Slide 41

Nuclear DNA barcoding for assessing the genetic identity of grapevine cultivars

✔ **Gene diversity descriptive statistics**

Overall number of polymorphic sites, observed and expected number of nucleotides per SNP site, information index of phenotypic diversity (SI) and polymorphism information content (PIC)

	No. A	P (%)	na	ne	SI	PIC
Italy	164	68 (62.4%)	1.67	1.19	0.201	0.140
Greece	22	39 (35.8%)	1.37	1.22	0.187	0.125
Portugal	32	41 (37.6%)	1.39	1.19	0.180	0.116
Rumania	8	33 (30.3%)	1.31	1.19	0.168	0.112
Spain	36	43 (39.4%)	1.42	1.23	0.195	0.128
Local	44	44 (40.4%)	1.42	1.17	0.169	0.106
Hybrid	12	56 (51.4%)	1.52	1.32	0.285	0.190
Outgroup	10	67 (61.5%)	1.74	1.40	0.361	0.123
Locals	44	44 (40.4%)	1.42	1.17	0.169	0.106
Cultivars	274	93 (85.3%)	1.94	1.21	0.219	0.134
Outgroups	10	67 (61.5%)	1.74	1.40	0.361	0.123
Total	313	108 (99.1%)	2.22	1.21	0.240	0.140
St. Dev.		14 (13.0%)	0.50	0.28	0.220	0.160

Summary of F-statistics and gene flow (Nm) estimates over all SNPs and accessions.

		F-statistics			
	n	Fis	Fit	Fst	Nm
Total	313	-0.09	0.10	0.18	1.12

RESULTS
Genetic diversity statistics were high for Italian cultivars whereas low genetic differentiation was found among cultivar subgroups (18%)

Gianni Barcaccia
Padua University

Orto Botanico di Padova – 3 Maggio 2011 41

Slide 42

Nuclear DNA barcoding for assessing the genetic identity of grapevine cultivars

✔ **Population structure analysis** (STRUCTURE v2.1)

RESULTS
A total of 4 distinct genetic clusters inferred out of the 8 sample subgroups predefined according to the geographical origin of cultivars
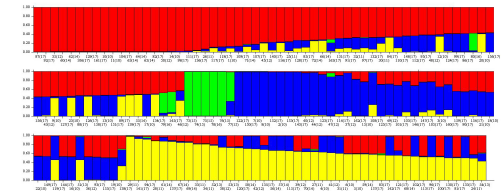
Admixed ancestry: 54% of accessions (q<0.7)

Subgroups	Inferred clusters				No. of individuals
	1	2	3	4	
Local	0.47	0.00	0.37	0.16	23
Italy	0.47	0.01	0.33	0.19	83
Greece	0.38	0.01	0.11	0.50	11
Portugal	0.35	0.01	0.42	0.22	16
Rumania	0.21	0.01	0.18	0.60	4
Spain	0.51	0.01	0.07	0.42	18
Hybrid	0.35	0.61	0.03	0.01	3
Outgroup	0.01	0.92	0.07	0.01	5

Gianni Barcaccia
Padua University

Orto Botanico di Padova – 3 Maggio 2011 42

Slide 43

Nuclear DNA barcoding for assessing the genetic identity of grapevine cultivars

✔ **Phenetic tree-building approach**
NJ tree showing relationships among *Vitis* species and *V. vinifera* cultivars

✔ **Genetic distances**
within (■) and between (■) accession groups

RESULTS
Construction of NJ trees based on the coalescence of specific populations

Gianni Barcaccia
Padua University

Orto Botanico di Padova – 3 Maggio 2011 43

Slide 44

Nuclear DNA barcoding for assessing the genetic identity of grapevine cultivars

✔ **Character-based approach**
detection of specific informative character states, SNPs or In/Dels, shared by taxonomic subgroups (biotypes and cultivars) and suitable for the identification of diagnostic haplotypes

1: AAACCTT
2: AAAYCTT
3: AAATCTT

• 1 SNP/34 bp
• Hn=127/152 CVs

RESULTS
Summary of SNP statistics for the nuclear single-copy barcode genes: all variable SNP sites were used (pi>95%)

Gene	Length (bp)	No. SNPs		Frequency (bp/1 SNP)		Hn	
		Vitis spp.	V. vinifera	Vitis spp.	V. vinifera	Vitis spp.	V. vinifera
GAI	761	18	15	42.27	50.73	23	18 (1-101)
ID04	419	21	20	19.95	20.95	33	28 (1-44)
IIC08	418	20	18	20.90	23.22	14	11 (2-86)
ATP	800	21	15	38.09	53.33	25	19 (1-73)
UFGT	919	29	29*	31.69	31.69	97	92 (1-12)
Combined	3317	109	97	30.43	34.19	132	127 (1-4)

Gianni Barcaccia
Padua University

Orto Botanico di Padova – 3 Maggio 2011 44

Slide 45

Nuclear DNA barcoding for assessing the genetic identity of grapevine cultivars

✔ **Multi-locus haplotypes**
(3 nuclear barcode regions)

Overall:
53 SNPs over 1,598 bp
(1 SNP/29 bp)

62 haplotypes
including 1 (38)
to 9 accessions

Replicated accessions:
• Sultanina
• Carmenere
• Malbech
• Cannonao
• Sagrantino
• Raboso piave

Synonymies:
• Merlot (cultivars vs. locals)
• Raboso piave vs. Friularo
• Marzemina (nera vs. cener.)

RESULTS
Multi-locus haplotype composition: heterozygous sites were dealt as functionally haploid SNPs (using IUB codes)

Gianni Barcaccia
Padua University

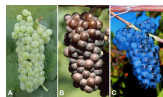**Orto Botanico di Padova – 3 Maggio 2011** 45

Slide 46

Nuclear DNA barcoding for assessing the genetic identity of grapevine cultivars

✔ **Single-locus haplotypes**
UFGT as barcode region: 919 bp
(1 SNP/32 bp)

92 haplotypes out of 152 cultivars including 1 to 12 accessions each

Cultivars/haplotype #
• 1 H : 1 CV   68
• 1 H : 2 CVs   13
• 1 H : 3 CVs   6
• 1 H : 4 CVs   1
• 1 H : >5 CVs   4

RESULTS
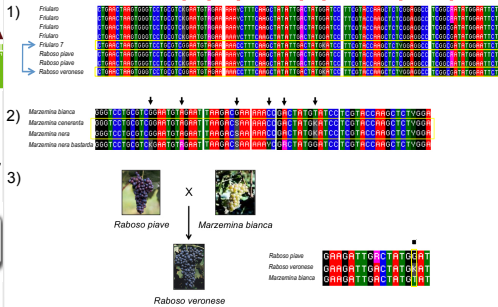Single-copy gene SNPs and haplotype composition

Gianni Barcaccia
Padua University

Orto Botanico di Padova – 3 Maggio 2011 46

Slide 47

Nuclear DNA barcoding for assessing the genetic identity of grapevine cultivars

✔ **Single-locus haplotypes**
UFGT as barcode region: 919 bp
(1 SNP/32 bp)

RESULTS
Single-copy gene SNP haplotype composition

92 haplotypes out of 152 cultivars including 1 to 12 accessions each

Gianni Barcaccia
Padua University

Orto Botanico di Padova – 3 Maggio 2011   47

Slide 48

Nuclear DNA barcoding for assessing the genetic identity of grapevine cultivars

✔ **Single-locus haplotypes**
UFGT as barcode region: 919 bp
(1 SNP/32 bp)

RESULTS
Single-copy gene SNP haplotype composition

92 haplotypes out of 152 cultivars including 1 to 12 accessions each

Gianni Barcaccia
Padua University

Orto Botanico di Padova – 3 Maggio 2011   48

Slide 49

Nuclear DNA barcoding for assessing the genetic identity of grapevine cultivars

✔ **Specific findings**
(mislabelling, synonymy/homonymy, hybrids)

1) Friularo / Friularo / Friularo / Friularo / Friularo 7 / Raboso piave / Raboso piave / Raboso veronese

2) Marzemina bianca / Marzemina cenerenta / Marzemina nera / Marzemina nera bastarda

3) Raboso piave X Marzemina bianca

Raboso piave / Raboso veronese / Marzemina bianca

Raboso veronese

FINDINGS
Grapevine DNA barcoding revealed cases of mislabeling, synonymy and hybridity

Gianni Barcaccia
Padua University

Orto Botanico di Padova – 3 Maggio 2011   49

Slide 50

Nuclear DNA barcoding for assessing the genetic identity of grapevine cultivars

Concluding remarks

✔ The chloroplast genome proved to be not enough polymorphic within *V. vinifera* species to distinguish grapevine cultivars;

✔ A multi-locus DNA barcoding based on five nuclear regions allowed to reconstruct cultivar-specific haplotypes (diagnostic characters fixed in single cultivars or cultivar subgroups):

• many cultivar-specific haplotypes were found;
• accessions belonging to a given cultivar clustered together sharing the same haplotype;
• several cases of homonymy/synonymy were resolved;
• the hybrid origin of some hybrids was verified;
• local cultivars were classified correctly with the international certified cultivars.

✔ The single-copy gene UFGT is an excellent candidate for the nuclear DNA barcoding of grapevine cultivars.

CONCLUSIONS
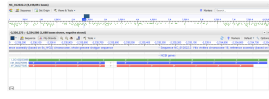Nuclear DNA barcoding opens new perspectives on the genetic traceability of grapevine cultivars

Gianni Barcaccia
Padua University

Orto Botanico di Padova – 3 Maggio 2011   50

Slide 51

Nuclear DNA barcoding for assessing the genetic identity of grapevine cultivars
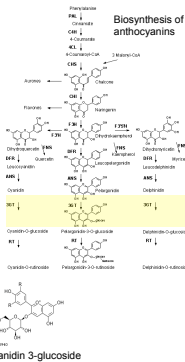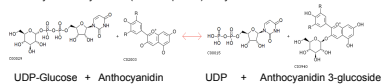
✔ **Information on VvUFGT**
Gene structure (1,536 nt, CDS=1,371 nt)

Protein (456 aa) domains and active sites

Biochemical function (glycosyl-transferase)
Anthocyanin biosynthesis (KEGG map00942) Enzyme 2.4.1.115

Biosynthesis of anthocyanins

UDP-Glucose + Anthocyanidin   UDP + Anthocyanidin 3-glucoside

UFGT
The best candidate gene for grapevine DNA barcoding

Gianni Barcaccia
Padua University

Orto Botanico di Padova – 3 Maggio 2011   51

Slide 52

DNA barcoding and its potentials for assessing genetic identity in plant species

Gianni Barcaccia
Padua University

Orto Botanico di Padova – 3 Maggio 2011   52