Campobasso, 21 Marzo 2007

La genomica per la valorizzazione delle risorse genetiche vegetali

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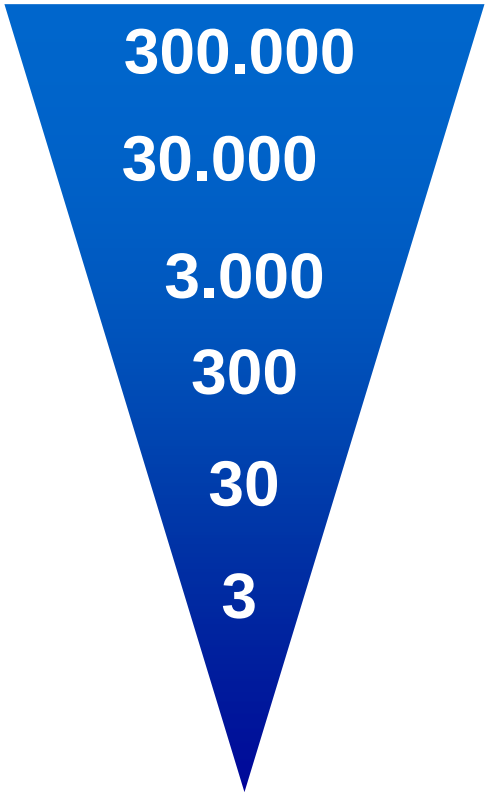
PLANT GENETIC RESOURCES



Plant species

Useful for humans

Used as food and other

Grown

Very important

60% of total plant calorie intake

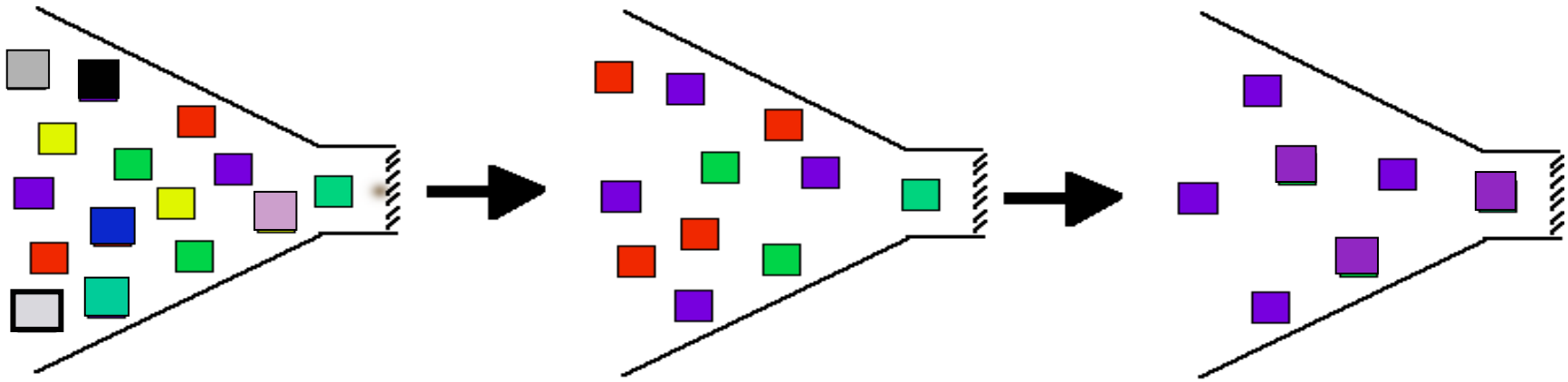
PLANT GENETIC COLLECTIONS

Crop	Total accessions world-wide	Crop	Total accessions world-wide
Wheat (*Triticum*)	784,500	Chickpea (*Cicer*)	67,500
Barley (*Hordeum*)	485,000	*Prunus*	64,500
Rice (*Oryza*)	420,500	Clover (*Trifolium*)	61,500
Maize (*Zea*)	277,000	*Capsicum*	53,500
Garden bean (*Phaseolus*)	268,500	Cotton (*Gossypium*)	49,000
Oat (*Avena*)	222,500	Grape (*Vitis*)	47,000
Soybean (*Glycine*)	174,500	*Triticale*	40,000
Sorghum	168,500	Alfalfa (*Medicago*)	33,000
Brassica	109,000	Sweet potato (*Ipomoea*)	32,000
Apple (*Malus*)	97,500	Potato (*Solanum tuberosum*)	31,000
Millet (*Eleusine*,*Panicum*)	90,500	Fava bean (*Vicia faba*)	29,500
Cowpea (*Vigna*)	85,500	Sunflower (*Helianthus*)	29,500
Groundnut (*Arachis*)	81,000	Lupin (*Lupinus*)	28,500
Tomato (*Lycopersicon*)	78,000	Cassava (*Manihot*)	28,000
Pea (*Pisum*)	72,000	Rye (*Secale*)	27,000

Total **4,038,000**

Hammer et al., 2003

GENETIC BOTTLENECKS IMPOSED ON CROP PLANTS

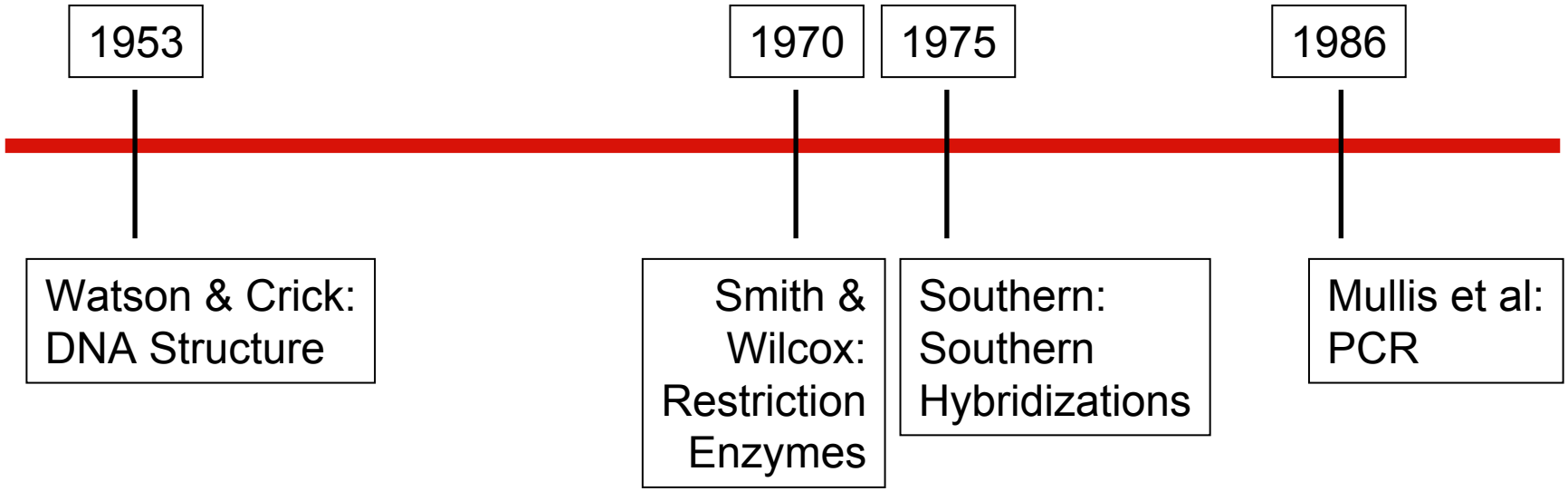


Tanksley & McCouch, 1997

Overcoming interspecific barriers: some examples

Tool	Crop	Traits transferred
Embryo culture	**(Tomato)** *S. lycopersicum* x *S. peruvianum*	Resistance to fungi and bacteria
2n gametes	**(Potato)** *S. tuberosum* (4x) x wild *Solanum* spp. (2x)	Resistance to fungi and frost
Ph gene	**(Wheat)** *T. durum/aestivum* x *Aegilops* and other *Triticeae*	Resistance to *Oidium* and rust Protein quality

GENOMICS MILESTONES



Restriction Enzymes / Ligase

RFLP
AFLP
Linkage mapping
Optical mapping
Library construction
Gene cloning
Tilling

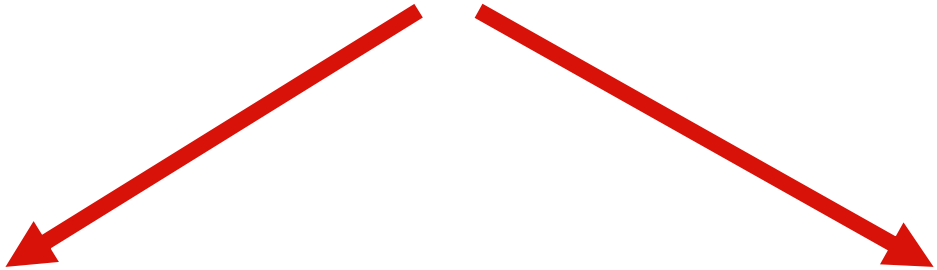
DNA:DNA Hybridization

Southerns / RFLP
Northerns
Microarrays
DArT markers
Taqman Real Time PCR
Chromosome painting

PCR

AFLP
RAPD
CAPS
SCAR
STS
Real Time PCR
RT-PCR
DNA sequencing
SNP detection

Genomics



Structural Genomics:

understanding how genomes are physically organized

- linkage and gene maps
- physical (BAC) maps
- DNA sequencing

Functional Genomics:

understanding how genomes and genes work

- transcriptomics
- transformation
- gene silencing

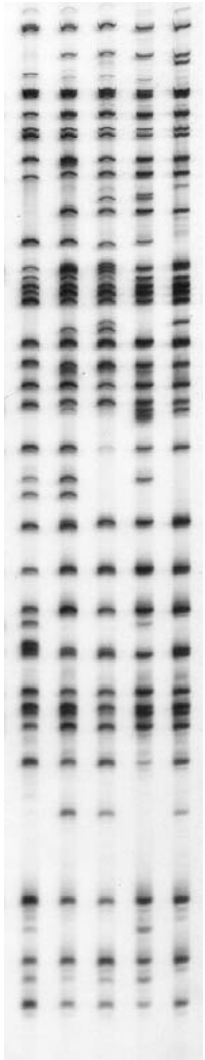
The impact of biotechnologies on the use of genetic resources

- Exploitation of genotypes
  (*Molecular markers*)
- Exploitation of single traits
  (*MAS*)
- Exploitation of single genes
  (*Forward & reverse genetics*)

The impact of biotechnologies on the conservation of genetic resources

	Conservation		
	Local varieties	Wild species	DNA
Exploitation of genotypes (*molecular markers*)	+		
Exploitation of single traits (*MAS*)	+	+	
Exploitation of single gene (*Forward & reverse genetics*)	+	+	+

IDENTIFICATION OF LOCAL VARIETIES



-Pomodoro “Vesuviano”
-Pomodoro “S. Marzano”
-Pomodoro “Sorrento”
-Pomodoro “Corbarino”
-Pomodoro “Africano”
-Pomodoro “Tondo di Sulmona”
-Pomodoro “A pera”
-Pomodoro “Grosso di Maria”

-Fagiolo “di Controne”
-Fagiolo “Occhio nero Oliveto Citra”
-Fagiolo “di Sarcione”
-Fagiolo “Poverello”
-Fagiolo “Marruzzo”
-Fagiolo “Verdolino”
-Fagiolo “Zolfino del Pratomagno”

-Nocciolo “Tonda di Giffoni”
-Nocciolo “Tonda delle Langhe”
-Nocciolo “Napoletanedda”
-Nocciolo “Riccia di Talanico”

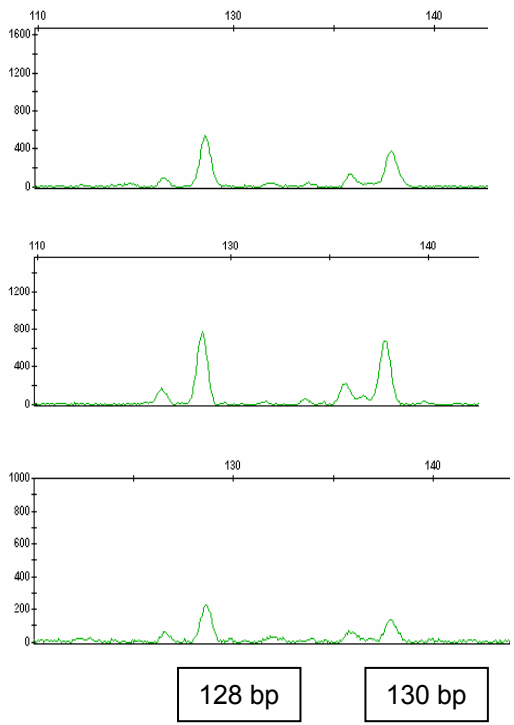
-Carciofo “Romanesco”
-Carciofo “Castellamare”
-Carciofo “ Spinoso di Sciacca”

GENETIC TRACEABILITY OF PLANT PRODUCTS WITH SSR MARKERS

OLIVE OIL

DNA source

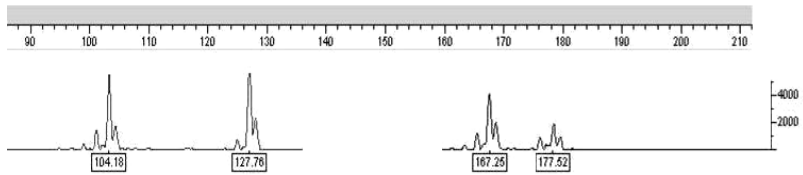
Allelic profile at locus DC4

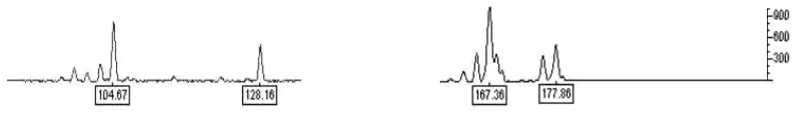


APPLE PRODUCTS

DNA source

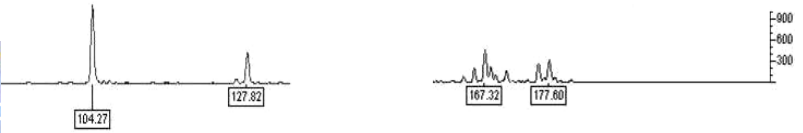
Allelic profile at 2 loci





purèe

nectar



CH01G12 **CH01F02**

Rao et al., 2006

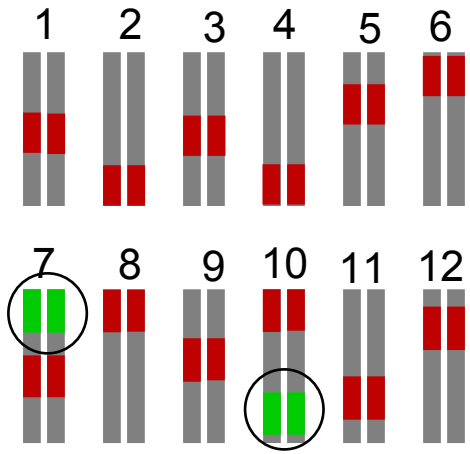
Molecular assisted selection: resistance genes pyramided in tomato

Cross	Pyramided genes	No. F2 analyzed	No. F4 homozygous at R loci
Momor x Motelle	*Frl, Tm2a, I2, Mi, Ve*	50	2
Momor x Ontario	*Frl, Tm2a, Pto*	30	4
Motelle x Ontario	*I2, Mi, Pto*	30	2
Okitzu x Ontario	*I2, Tm2a, Pto*	30	6
Okitzu x Pyrella	*I2, Tm2a, py-1*	30	2

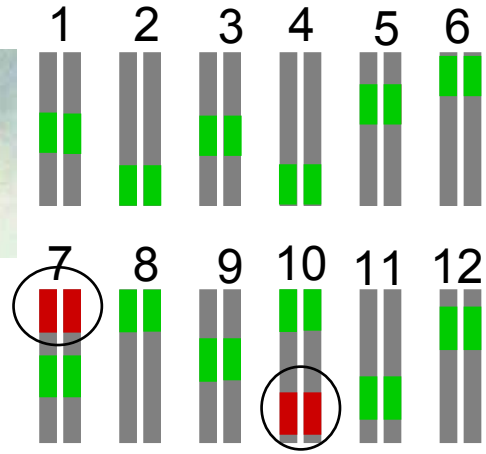
Barone et al., 2004

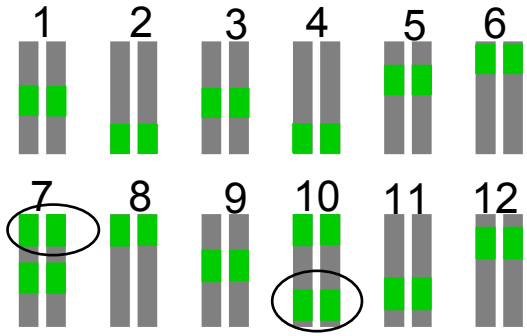
Use of MAS to transfer useful QTLs from wild species to the cultivated tomato

Wild species chromosomes



Cultivated tomato chromosomes





Inbred line

Grandillo et al. 2000

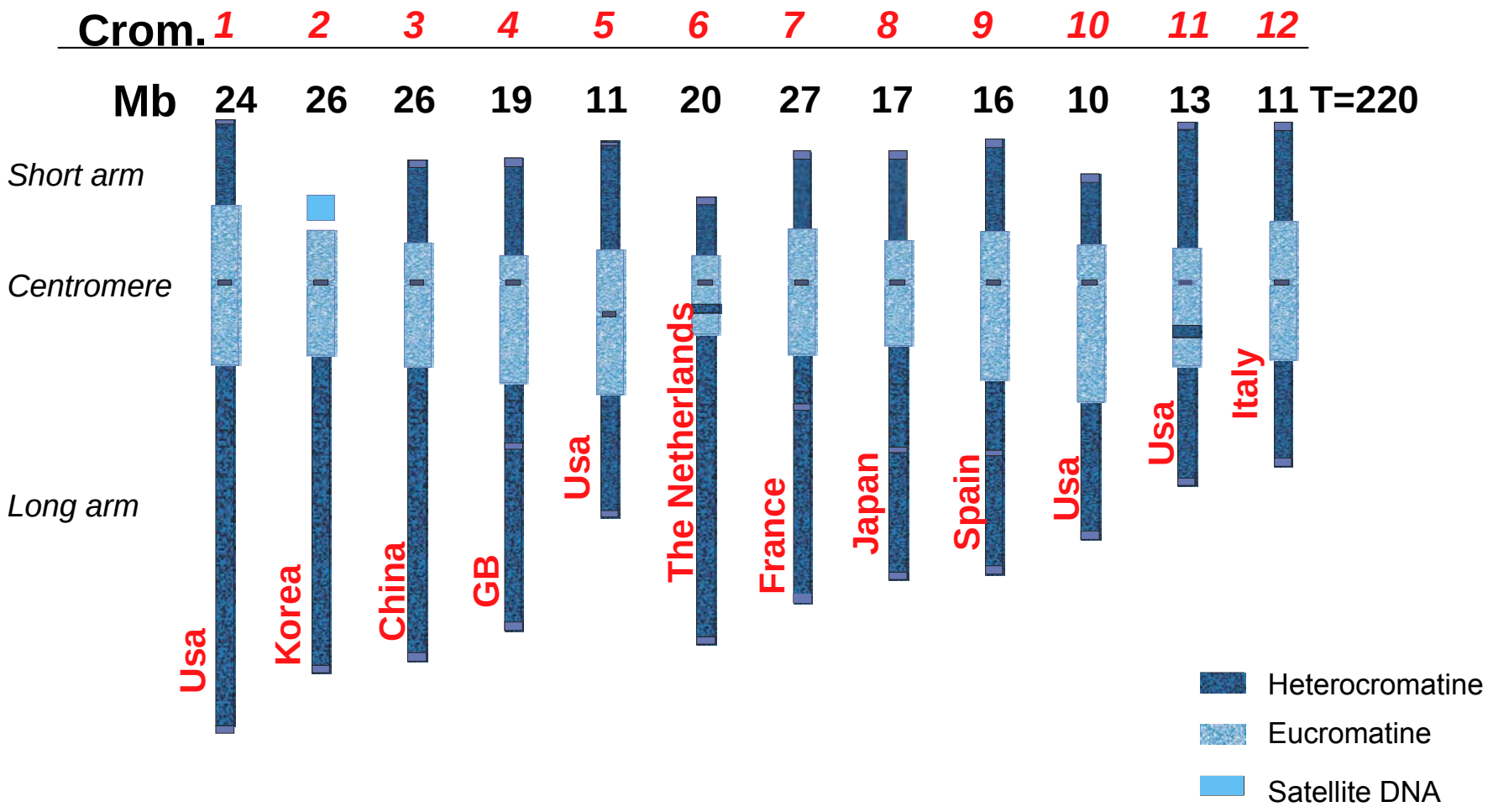
Plant nuclear genome sequences available

- Arabidopsis thaliana (Nature 408, 769-815)
- Oryza sativa ssp. japonica (Science, 269, 92-100)
- Oryza sativa L. ssp. indica (Science 296, 79-92)

Sequencing projects under way

- ✓*Medicago truncatula*
- ✓*Lotus japonica*
- ✓*Solanum lycopersicum*
- ✓*Solanum tuberosum*
- ✓*Zea mais*
- ✓*Brassica oleracea*
- ✓*Hordeum vulgare*
- ✓*Glycine max*
- ✓*Musa spp.*
- ✓*Populus trichocarpa*

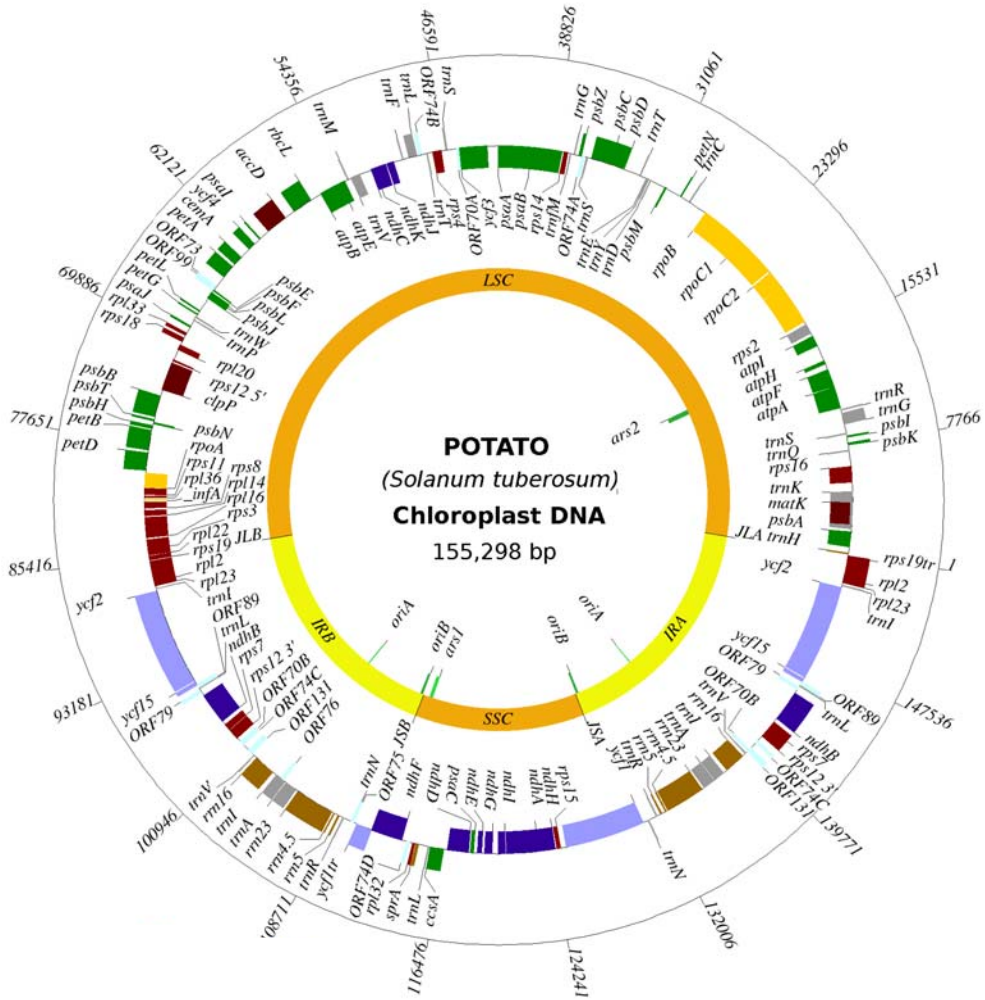
The tomato genome sequencing project



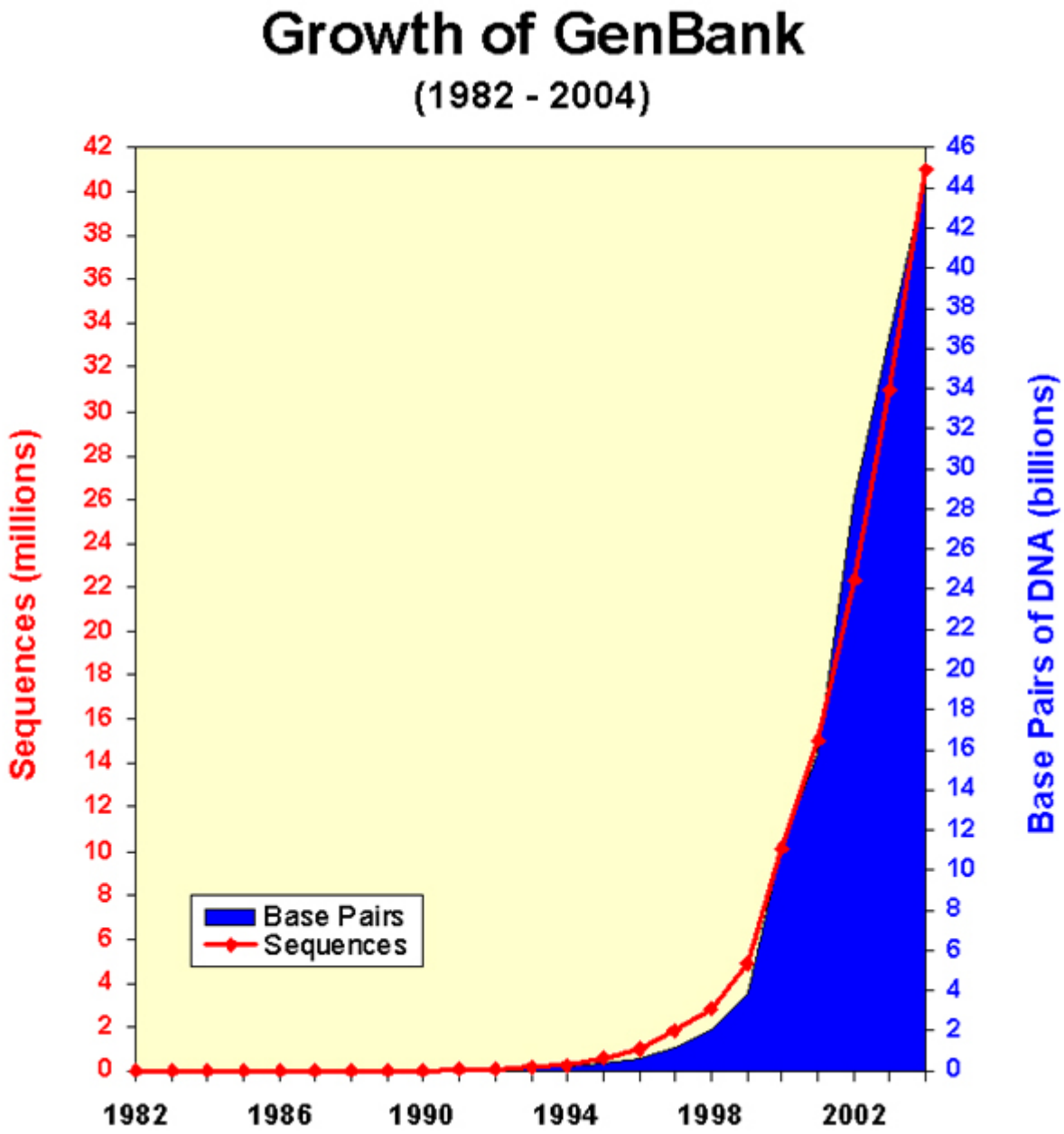
Cytoplasmic DNA sequences are available

- RNA ribosomal genes
- Transfer RNA genes
- Ribosomal protein genes
- Transcription/translation apparatus genes
- Photosynthetic apparatus genes
- NADH dehydrogenase genes
- Other protein genes
- Conserved reading frames
- Open reading frames

The potato (cv. Desiree) plastome



Cardi et al., 2005


Growth of GenBank
(1982 - 2004)
Sequences (millions)
Base Pairs of DNA (billions)
Base Pairs
Sequences
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40
42
44
46
1982
1986
1990
1994
1998
2002

The Italian Plant DNA Bank: towards an integrated system for conservation and utilization of genetic resources

CNR-IGV (project leader Gabriella Sonnante)

A DNA bank is an extension of the concept of gene-bank initially implemented in seed genebanks and is not meant as a substitute.

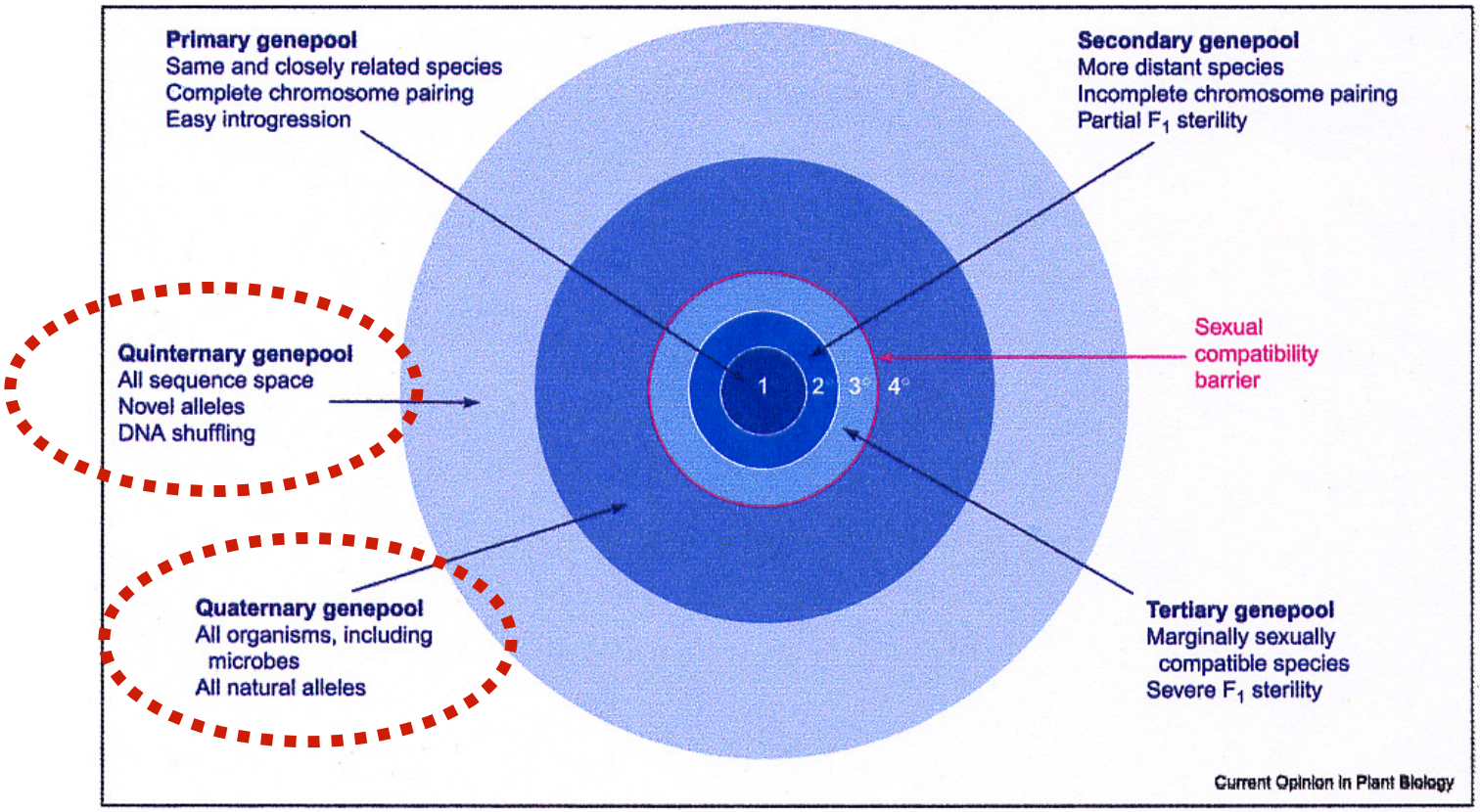
Why a DNA bank?

- Monitoring changes in populations genetic structure
- Assessment of genetic diversity
- Disclose taxonomicrelation ships
- Search for new gene variants
- Development of new markers
- Identification and traceability of typical products
- Fraud prevention tools
- DNA conservation on request
- DNA distribution

What to store?

- Landraces of species of interest (e.g.: Italian tomato landraces)
- Typical products (e.g.: Peperone di Senise PGI)
- Crop wild relatives (e.g.: wild artichoke)
- Wild plants used by man (*Eruca*, *Borago*, etc.)
- Model plants (*Arabidopsis thaliana*, *Medicago truncatula*)
- Isolated genes, clones, mutants, etc.
- Specific DNA markers

Genomics has expanded from 3 to 5 the number of gene pools



Michelmore, 2003

Resistance genes isolated and cloned in tomato whose sequence is available in GenBank

GENE	PATHOGEN	CHROMOSOME
Asc-1	*Alternaria alternata* f.sp. *lycopersici*	3
Bs4	*Xanthomonas campestris* pv *vesicatoria*	5
Cf2, Cf4, Cf5, Cf9	*Cladosporium fulvum*	1, 6
Hero	*Globodera rostochiensis*	4
I2	*Fusarium oxysporum* f sp *lycopersici*	11
Mi *	*Meloidogyne* spp	6
Pto	*Pseudomonas syringae* pv tomato	5
Sw5	TSWV	9
Tm2a	TMV	9
*Ve1, Ve2***	*Verticillium dahliae*	9

* confers resistance also to potato aphids

** confer resistance also to *Verticillium* in potato

NEW APPROACHES FOR TRANSGENIC PLANT PRODUCTION

- Use of plant genes and promoters
  (e.g.: antisense RNA)
- Elimination of marker genes
- Direct transformation
  (e.g.: gene-gun, electroporation)
- *In vivo* transformation
  (no somaclonal variation)

Caratteri presenti in piante transgeniche in commercio

CARATTERE	GENE UTILIZZATO	EFFETTO GENE INSERITO
Resistenza ad insetti	Cry e Bt (*Bacillus thuringensis)*	Proteine tossiche per gli insetti
Tolleranza ad erbicidi	EPSPS (pianta)	Insensibilit□ all□erbicida glifosato
	PAT (batterio)	Insensibilit□ all□erbicida gufosinate ammonio
	BXN(batterio	Resistenza all□erbicida bromoxynil
	Bar (batterio)	Insensibilit□ all□erbicida gufosinate ammonio e fosfinotricina
Resistenza a virus	CP (virus)	Resistenza al virus Y della patata
	RNA antisenso	Interferenza con la replicazione del virus
	Fny (replicasi mutata del virus CMV)	
Maschiosterilit	Barnase (batterio)	Maschiosterile e resistente ad erbicidi
	Barnase/Barstar (batterio)	Distruzione cellule del tappeto dell□antera e ristorazione maschiosterilit□
Colore del fiore	ACC sintasi antisenso (pianta)	Blocco della sintesi dell□etilene
	DF reduttasi antisenso (pianta)	Blocco della sintesi degli antociani
Contenuto di acidi grassi	_ _____	Aumento del contenuto di acido oleico

Caratteri presenti in piante transgeniche in sperimentazione

CARATTERE	GENE UTILIZZATO	EFFETTO GENE INSERITO
Resistenza a funghi	RIP (pianta)	Proteine che inattivano la sintesi proteica
	Osmotina (pianta)	Resistenza a funghi
Resistenza a carenza idrica	Mannitolo 1P- deidrogenasi	Accumulo di mannitolo
Miglioramento qualit frutto	Poligalatturonasi antisenso (pianta)	Blocco della degradazione della pectina
	Fruttosiltrasferasi (batterio)	Aumento del contenuto di fruttani
Miglioramento valore nutrizionale	Fitoene sintasi e Licopene _ ciclasi (pianta)	Aumento della produzione di provitamina A
	Saccarosio fosfato sintasi	Accumulo di zucchero
Composti farmaceutici	Ormone della crescita (uomo)	Sintesi in pianta di composti farmaceutici
	Eritropoietina (uomo)	
	Collagene (uomo)	
Anticorpi ricombinanti	IgG ed IgM	Sintesi in pianta di anticorpi
Produzione di vaccini	Tossina del colera, tetano e rotavirus	Sintesi in pianta di vaccini
	Proteina ÒsÓ envelope del virus dellÕepatite B	
	Proteina del capside del virus Norwalk	
	Autoantigene per il diabete	