UNIVERSITÀ DEGLI STUDI DELLA Tuscia
DIBAF
Dipartimento per la Innovazione nei sistemi Biologici, Agroalimentari e Forestali

Consiglio Nazionale delle Ricerche (CNR)
Istituto di Biologia Agroambientale e Forestale (IBAF)
Via Salaria Km. 29,300
00016 Monterotondo Scalo (Roma), ITALY

Risposta adattativa di alberi forestali e miglioramento genetico

Sabatti M.[1], **Beritognolo I.**[2], **Villani F.**[2], **Malvolti M. E.**[2]

[1] Dipartimento per l'Innovazione nei sistemi Biologici Agroalimentari e Forestali. Università degli Studi della Tuscia. Viterbo
[2] Istituto di Biologia Agro-ambientale e Forestale. Consiglio Nazionale delle Ricerche. Porano (TR)

Esperienze e prospettive del miglioramento genetico degli alberi forestali in Italia
Consiglio per la Ricerca e l'analisi dell'Economia Agraria – Azienda Ovile
Roma, 30 maggio 2018

NovelTree
SEVENTH FRAMEWORK PROGRAMME

Experiences on fast growth and multipurpose forest species

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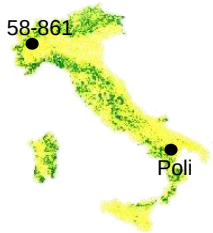
Poplars
Fast growth,
Biomass,
Wood,
Agro-forestry systems
Ecosystem services

Chestnut
Multipurpose,
Fruits,
Food,
Agro-forestry systems
Ecosystem services

Walnut
Multipurpose
Fruits,
Wood,
Agro-forestry systems
Ecosystem services

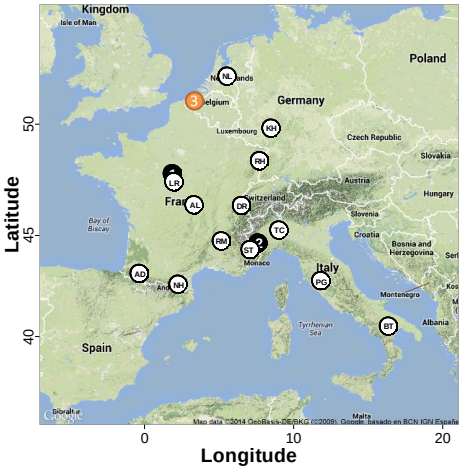
F_1 *Populus nigra* family (POP5)



162 clones planted in:
Cavallermaggiore (CAV, 2004)
Savigliano (SAV, 2008)
Viterbo (VT, 2008)
Montelibretti (MT, 2004)

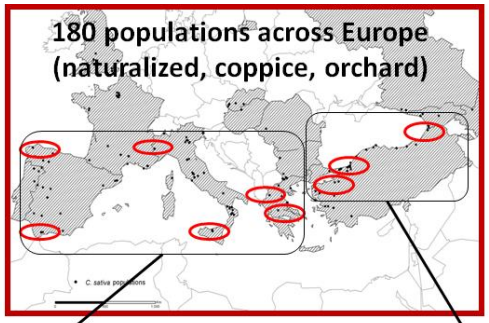
J. regia ecotypes

***P. nigra* association population**

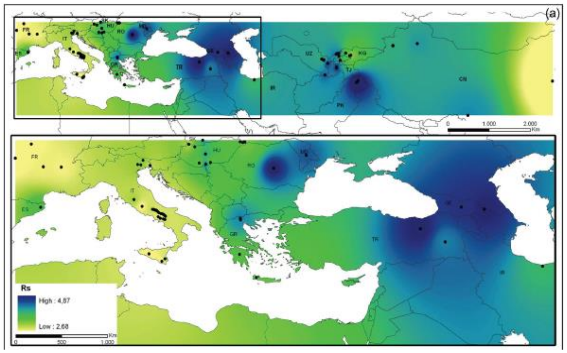


Geographic origin of 13 metapopulations of the *Populus nigra* collection (white circles) and geographic position of the three field trials: ORL (1) and SAV (2) (black circles), GDB (3) (red circle).

***Castanea sativa* populations & full sib families**



***Juglans regia* allelic richness across Europe**

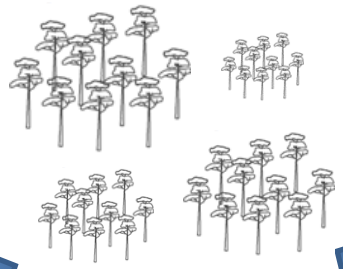


Full sib families

Grafting

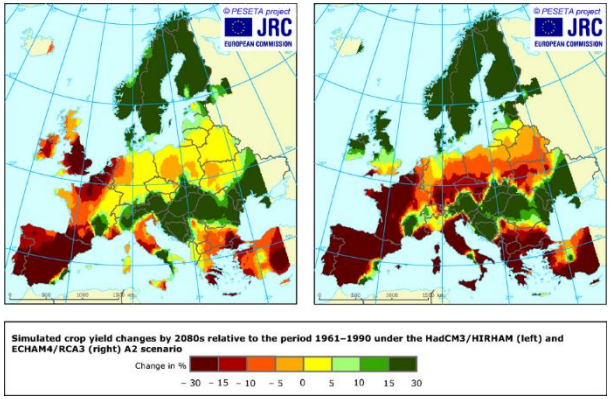
J.regia varieties

Future challenges of tree genetic improvement

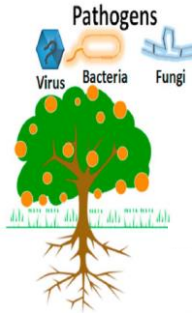


Target species

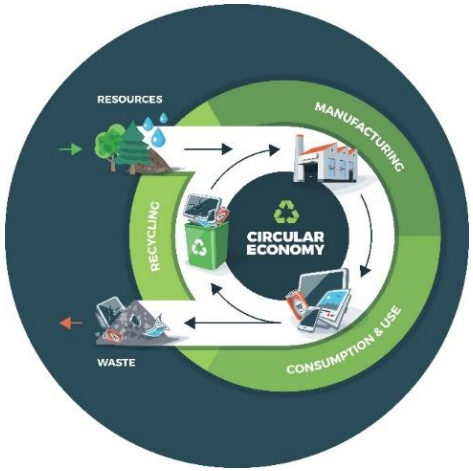
Changing climate



Changing threats



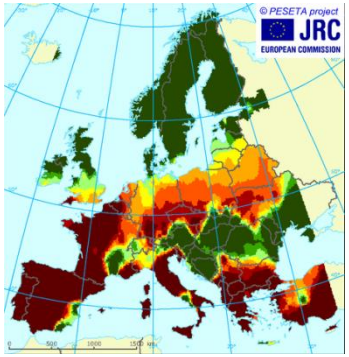
Changing products



Breeding for long lived species

Requests driven by changing environmental, productive and societal needs

General objectives:

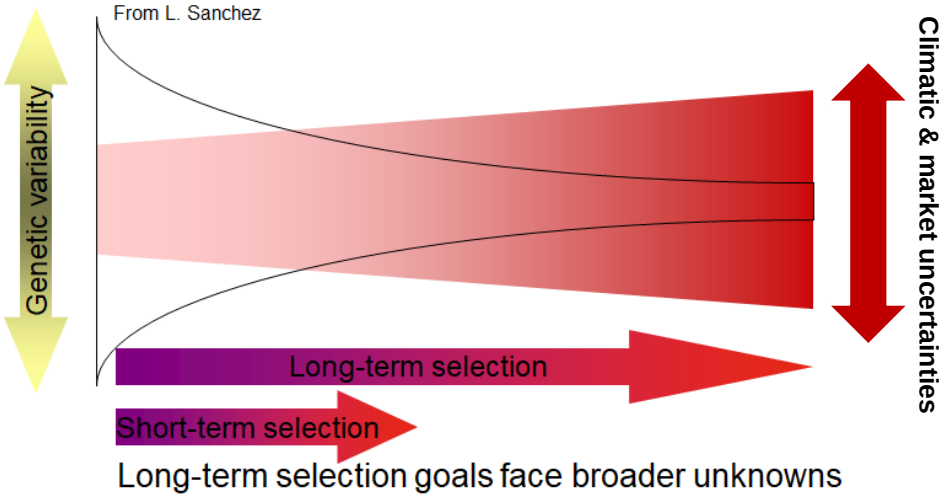


Management and conservation
of genetic resources

Adaptability
stability of performances in changing environment

Specific added values
(new markets, circular economy)

Continuous genetic gains
for wood and fruit production



➢ ***Multidisciplinary research activities at different scales:*** ***gene, individual, population, landscape***

A common parameter: Genetic Variability

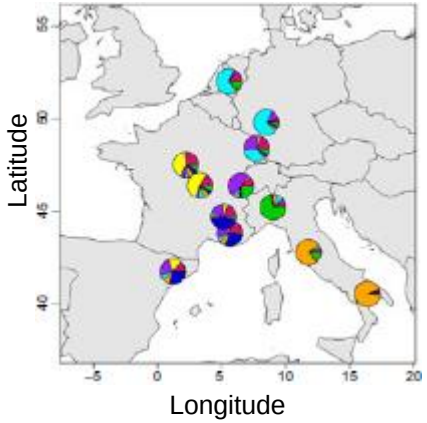
Wide geographic range and high adaptive potential

Populus nigra

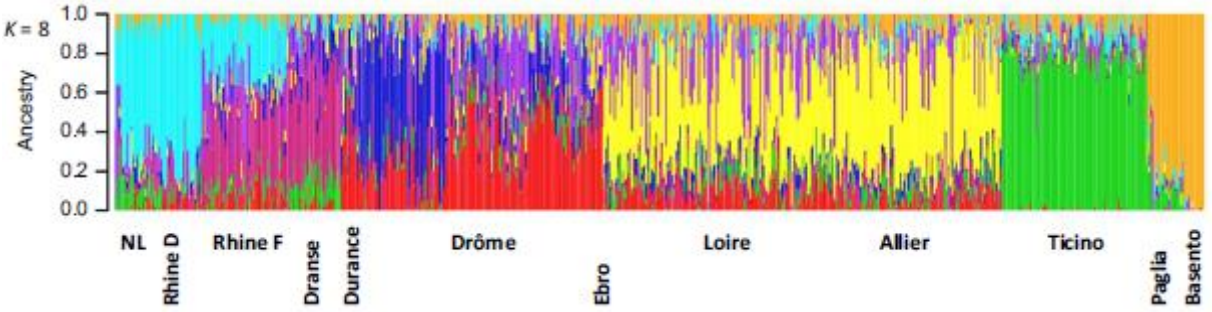
MOLECULAR ECOLOGY
RESOURCES
Molecular Ecology Resources (2016) 16, 1023–1036 doi: 10.1111/1755-0998.12513

New resources for genetic studies in *Populus nigra*: genome-wide SNP discovery and development of a 12k Infinium array

P. Faivre Rampant et al. 2016



Geographical distribution of the populations and the genetic structure revealed by ADMIXTURE.



Population structure analysis estimated from 600 SNPs distributed throughout the Populus nigra genome in validated genotypes.

- Seven gene pools across the *P. nigra* Western range
- High level of admixture, clear genetic differentiation between populations
- Italian populations structured along a latitudinal gradient, low level of admixture.

A common parameter: Genetic Variability

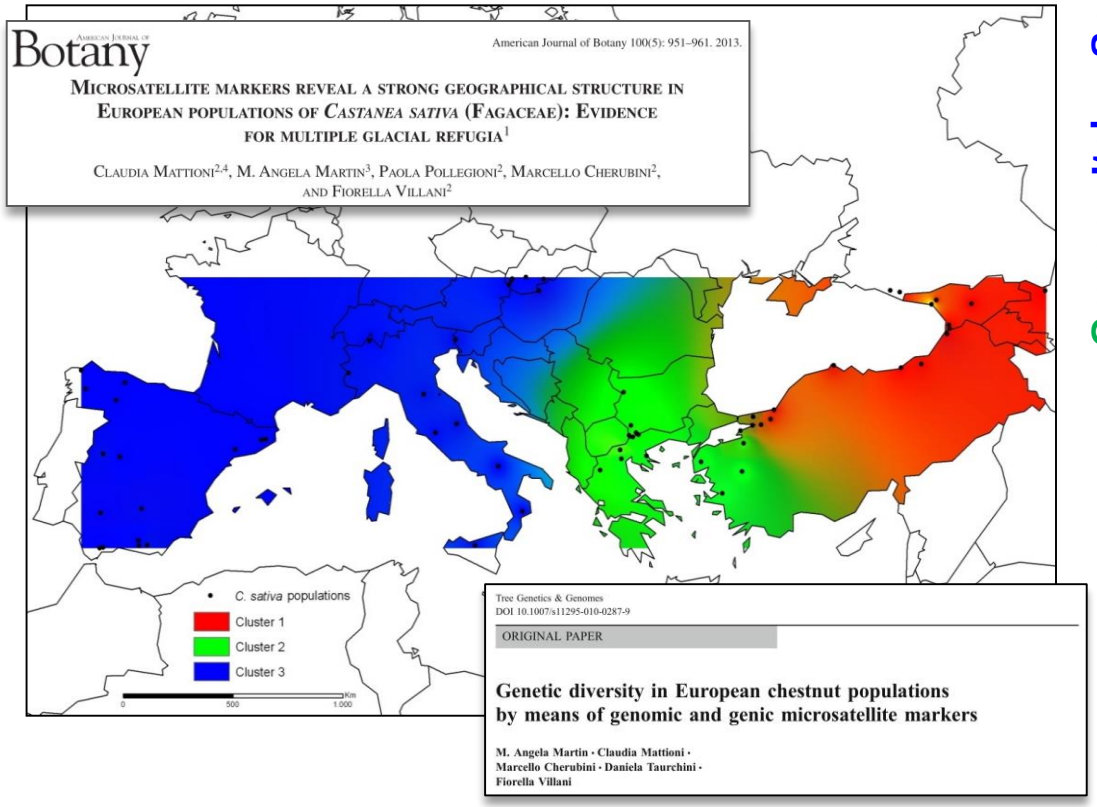
Wide geographic range and high adaptive potential

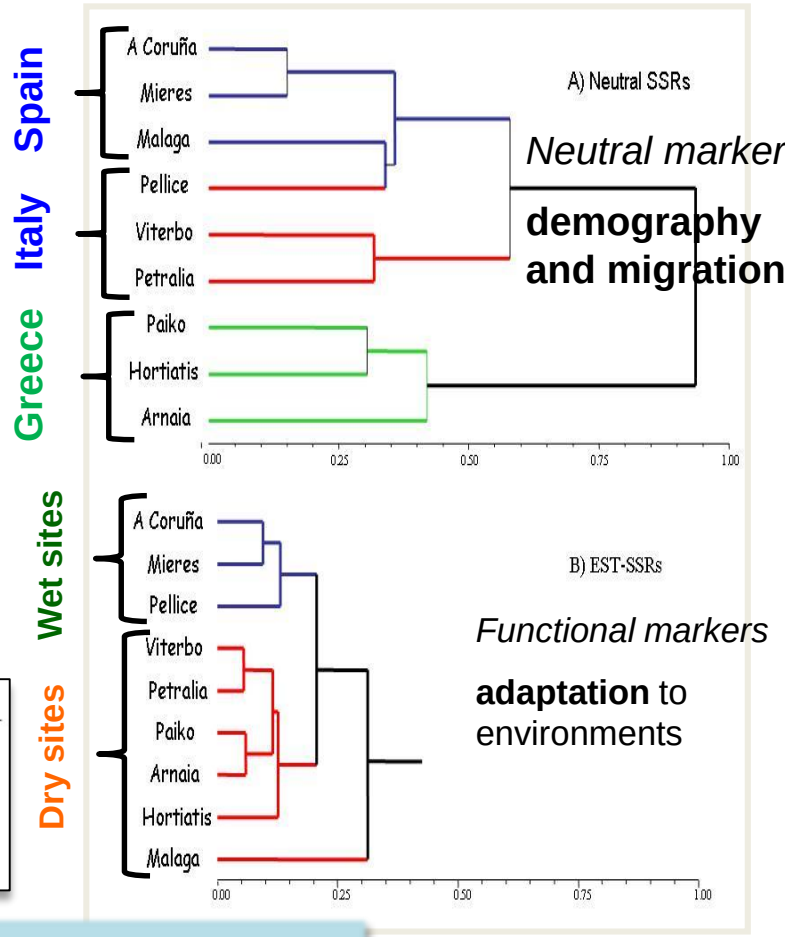
Castanea sativa

Genetic resources: genetic diversity

Genetic structure of populations

Main gene pools of *C. sativa* natural populations in Europe
Neutral diversity (SSRs)





- Three main gene pools across *C. sativa* natural range
- Different population genetic structuring: history vs. adaptation

A common parameter: Genetic Variability

Juglans regia

Wide geographic range and high adaptive potential

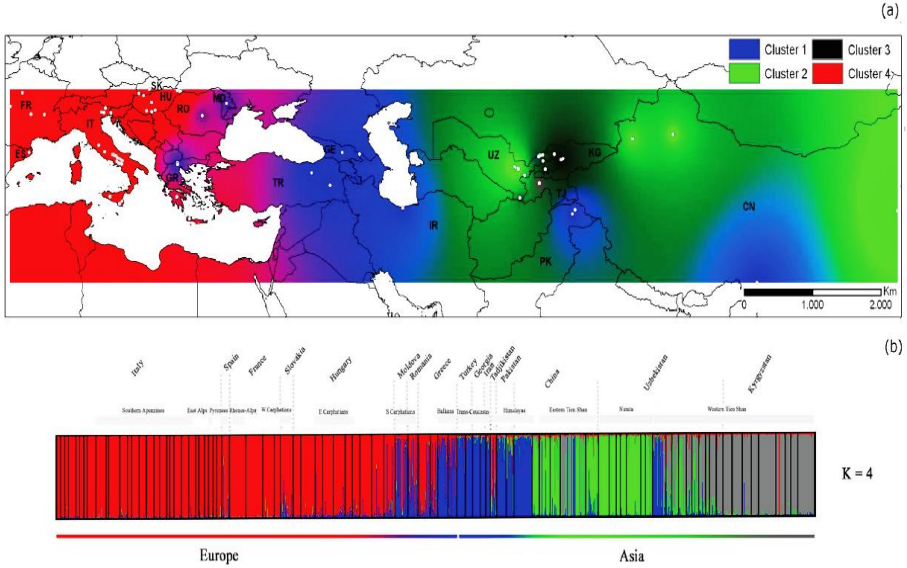
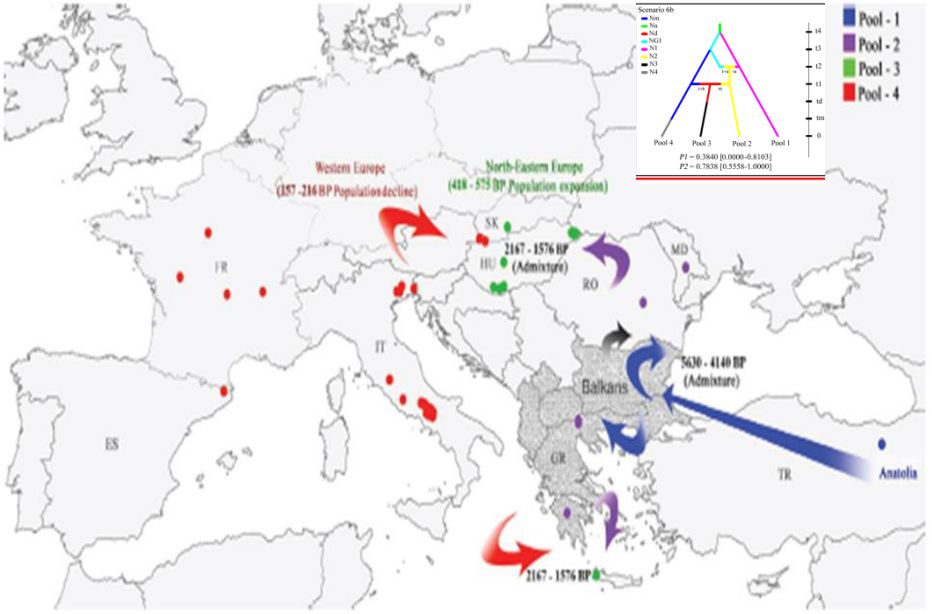


Fig 2. Spatial genetic structure of 91 walnut populations in Eurasia. Population structure inference for 91 walnut populations by Bayesian assignment using STRUCTURE for K = 4. Synthetic map of Inverse Distance Weighted (IDW) interpolations of the estimated mean population membership values (Q_i) (a) and bar plot showing assignment probabilities of individuals to K clusters (b). Abbreviations: CN = China, UZ = Uzbekistan, KG = Kyrgyzstan, TJ = Tajikistan, PK = Pakistan, IR = Iran, GE = Georgia, TR = Turkey, MD = Moldova, RO = Romania, HU = Hungary, SK = Slovakia, GR = Greece, IT = Italy, FR = France, ES = Spain.

Natural and human-mediated evolutionary history



Human-mediated dispersal routes of walnut during the Late Holocene as inferred by approximate Bayesian computation . Arrows represent the relationships between population pools used in DIYABC analysis (Pool 1, Pool 2, Pool 3, Pool 4) as inferred from stage 2, scenario 6b.

Tree Genetics & Genomes
DOI 10.1007/s11295-014-0740-2

ORIGINAL PAPER

Landscape genetics of Persian walnut (*Juglans regia* L.) across its Asian range

Paola Pollegioni · Keith E. Woeste · Francesca Chiocchini · Irene Olimpieri · Virginia Tortolano · Jo Clark · Gabriel E. Hemery · Sergio Mapelli · Maria Emilia Malvolti

Acta Hortic. 1190. ISHS 2018. DOI 10.17660/ActaHortic.2018.1190.5
Proc. Int. Symp. on the Role of Plant Genetic Resources in Reclaiming Lands and Environment Deteriorated by Human and Natural Actions
Eds.: A. Gharaghani and M. Khosh-Khui

PLOS ONE

RESEARCH ARTICLE

Ancient Humans Influenced the Current Spatial Genetic Structure of Common Walnut Populations in Asia

Paola Pollegioni[1]*, Keith E. Woeste[2], Francesca Chiocchini[1], Stefano Del Lungo[3], Irene Olimpieri[1], Virginia Tortolano[1], Jo Clark[4], Gabriel E. Hemery[5], Sergio Mapelli[6], Maria Emilia Malvolti[1]

PLOS ONE

RESEARCH ARTICLE

Rethinking the history of common walnut (*Juglans regia* L.) in Europe: Its origins and human interactions

Paola Pollegioni[1]*, Keith Woeste[2], Francesca Chiocchini[1], Stefano Del Lungo[3], Marco Ciolfi[1], Irene Olimpieri[1], Virginia Tortolano[1], Jo Clark[4], Gabriel E. Hemery[5], Sergio Mapelli[6], Maria Emilia Malvolti[1]

Adaptive variation: the study of growth phenology

Populus nigra

Bud flush in the *P. alba* common garden study at Viterbo – Italy

Timing of bud flush and bud set represent a critical ecological and evolutionary tradeoff between survival and growth.

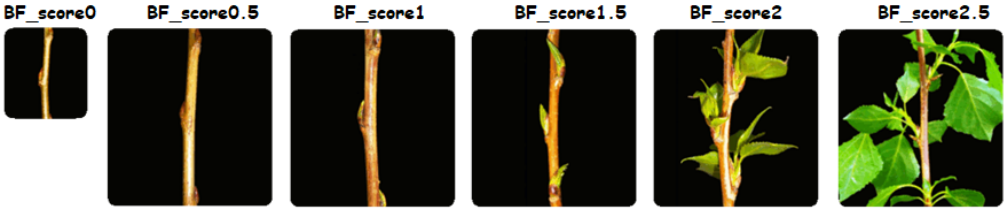


Figure 2. High-resolution scoring system for bud flush process in *Populus nigra*. Pictures were taken from individuals of *P. nigra* (Ludovisi, 2014). The scoring system for bud flush process was adapted and modified from Castellani et al. (1967).

Bud flush: depends mainly on the cumulative temperature sum in spring

Bud set: day length is the critical signal for growth cessation even if the timing of bud formation is also influenced by other factors such as:

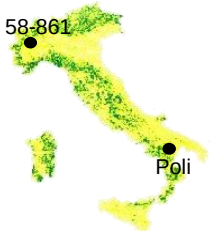
- temperature
- temperature × photoperiod
- nutrition
- drought
- biotic stresses.

Figure 3. High-resolution scoring system for bud set process in *Populus nigra*. Pictures were taken from individuals of *P. nigra* (Fabbrini et al., 2012). The scoring system for bud set process was adapted and modified from Rohde et al. (2011).

Adaptive variation: bud set in the *P. nigra* pedigree

Populus nigra

F_1 *Populus nigra* family (POP5)



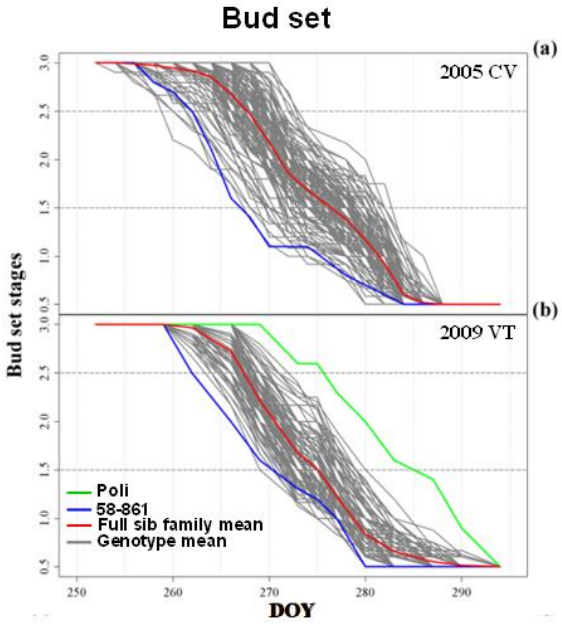
Segregation confirmed important additive effects inherited from 58-861 and Poli parents
→ a favourable situation to identify QTL

Fabbrini *et al. BMC Plant Biology* 2012, **12**:47
http://www.biomedcentral.com/1471-2229/12/47

BMC Plant Biology

RESEARCH ARTICLE Open Access

Phenotypic plasticity, QTL mapping and genomic characterization of bud set in black poplar

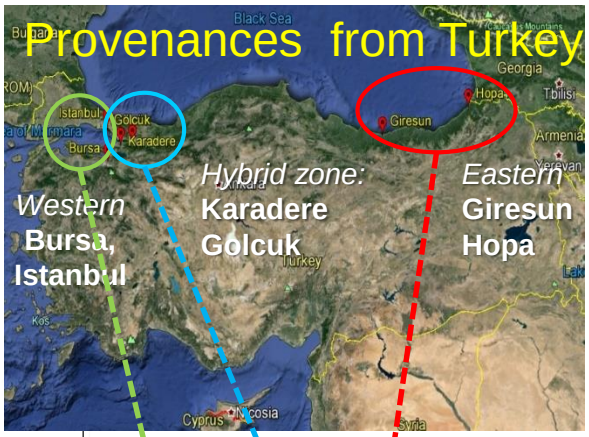


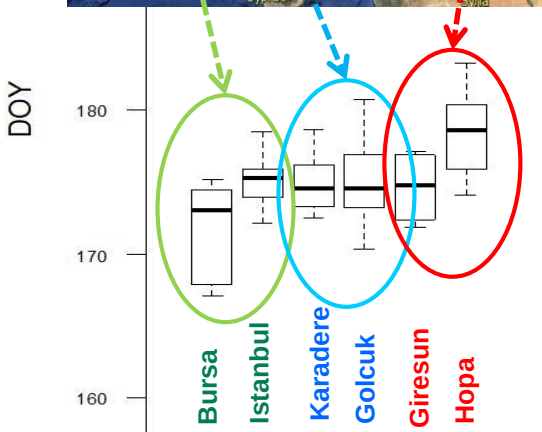
✓ High phenotypic variation in F_1 family (range 20 days) and moderate heritability (H^2 = 0.45).
✓ Considerable genotype × environment (G × E) interaction in all phenological stages, low temperatures influence the sensitivity of the most plastic genotypes.

Adaptive variation: floral phenology in chestnut

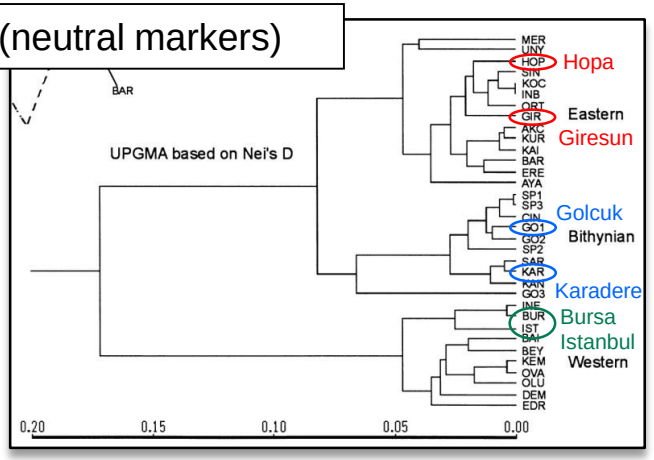
Castanea sativa

Common garden studies on natural germplasm

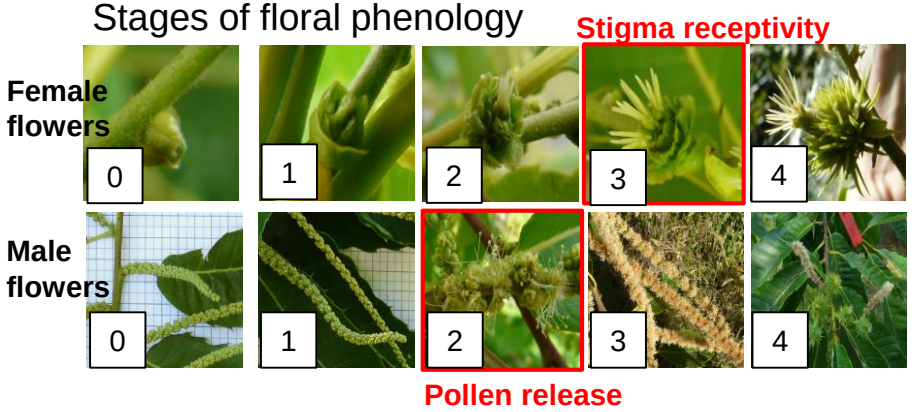




Genetic distance (neutral markers)



Stages of floral phenology



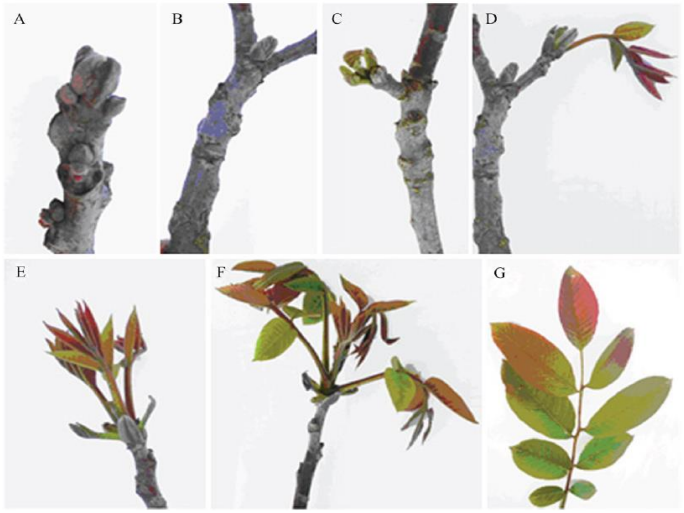
- Phenology variation consistent with geographic and genetic distance:
  western provenances express earlier flowering than eastern provenances

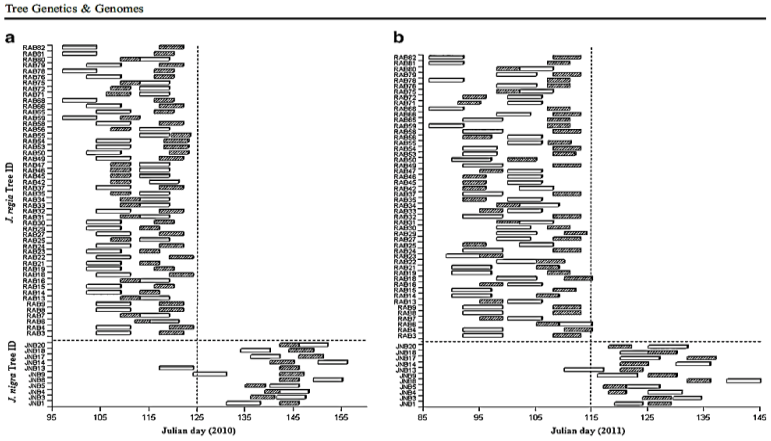
Adaptive variation: floral phenology in walnut

Juglans regia

Genetic improvement and breeding

(Twenty years of cooperation with CREA FL and CD)





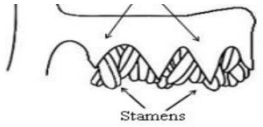
Flowering period of female (white bars) and male (dashed bars) inflorescences for 11 Juglans nigra (JNB1-JNB20) and 50 Juglans regia (RAB3-RAB82) adult trees in 2010 (a) and 2011 (b).

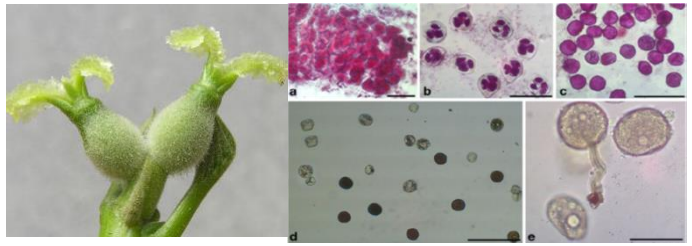
Tree Genetics & Genomes (2013) 9:291–305
DOI 10.1007/s11295-012-0555-y

ORIGINAL PAPER

Barriers to interspecific hybridization between *Juglans nigra* L. and *J. regia* L species

Paola Pollegioni · Irene Olimpieri · Keith E. Woeste · Giovanni De Simoni · Maria Gras · Maria E. Malvolti

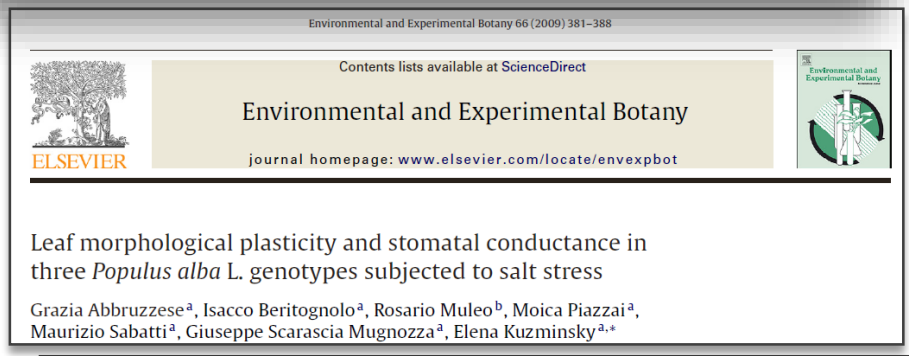


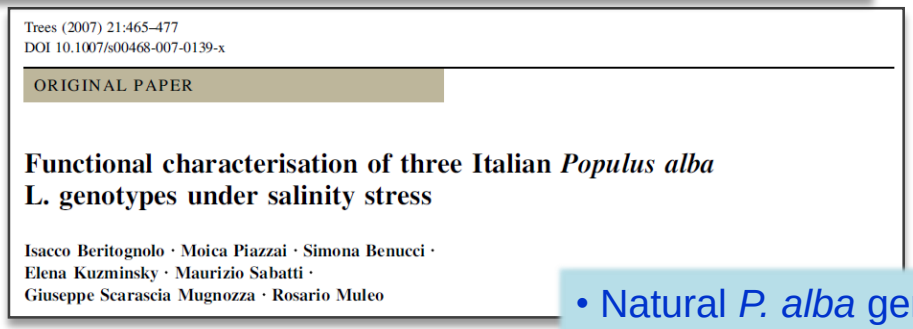


Morphological characters of receptive pistillate flowers and mature catkins collected from 11 Juglans nigra (empty square) and 50 Juglans regia (filled square) adult trees ovary size (mean length of equatorial diameter A and polar diameter B±SD), mean length of style (±SD), stigma (±SD), and catkin (±SD). Statistical significance was tested by Student's t test with∗∗p<0.0001

Abiotic stress: response to salinity in *P. alba*

Populus alba





- *P. alba* provenances from contrasting sites
- Comparative experiments (ex situ)

Differential mechanisms control variability in salinity tolerance

- **Salt exclusion in roots**
- **Salt compartimentalization in cells**
- **Plasticity of leaf micromorphology**
- **Stomatal regulation**
- **Transcriptome regulation**

Candidate genes

Tonoplast proton pumps
Heat Shock Proteins (HSP70)
Trehalose-6-phosphate synthase

- Natural *P. alba* genetic resources to improve salinity tolerance
- Selection of functional molecular markers to aid breeding

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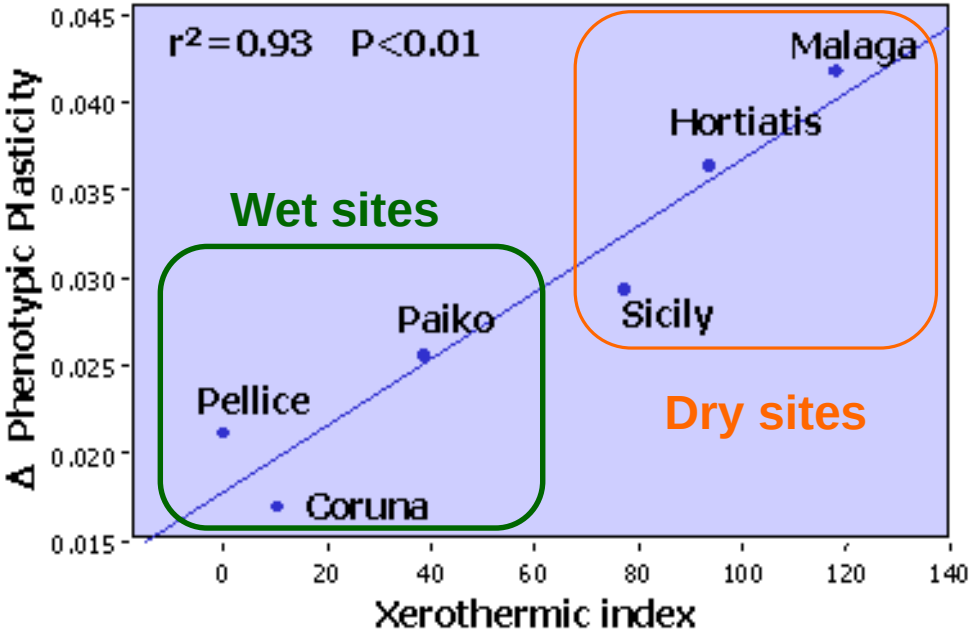
Abiotic stress: drought tolerance in *C. sativa*

Castanea sativa

Phenotypic plasticity of Water Use Efficiency correlates with Xerothermic Index



Genetic variation in carbon isotope discrimination in six European populations of *Castanea sativa* Mill. originating from contrasting localities

M. LAUTERI,* A. PLIURA,† M. C. MONTEVERDI,* E. BRUGNOLI,* F. VILLANI* & G. ERIKSSON‡

*Consiglio Nazionale delle Ricerche, Istituto di Biologia Agroambientale e Forestale, Porano, Italy
†Department of Forest Genetics and Reforestation, Lithuanian Forest Research Institute, Girionys, Lithuania
‡Department of Forest Genetics, SLU, Uppsala, Sweden

J. EVOL. BIOL. **17** (2004) 1286–1296

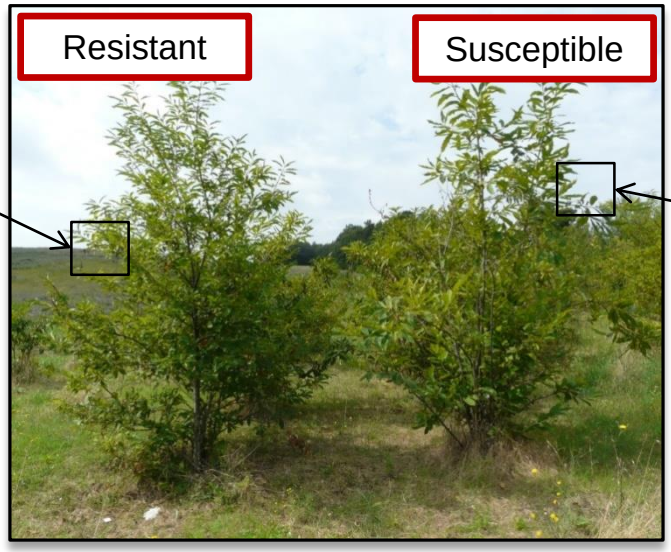
- Delta $_{13}C$ /$_{12}C$ isotope discrimination of leaves

• Provenances from dry sites express a higher phenotypic plasticity in response to drought
• Phenotypic plasticity contributes to environmental adaptation

Biotic stress: chestnut gall wasp

Castanea sativa

Susceptibility to *Drycosmus kuriphylus* in common garden provenance trial (CNR IBAF)



Infestation of buds 2016

Provenance	Sampled plants	Resistant plants	% of resistant
Coruna (SPAIN) wet	19	0	0
Malaga (SPAIN) dry	21	1	4.8
Pellice (ITALY) wet	17	0	0
Petralia Sottana (ITALY) dry	20	0	0
Paiko (GREECE) wet	20	3	**15**
Hortiatis (GREECE) dry	20	6	**30**

- Greek provenances:
  - high percentage of resistant plants
  - Valuable genetic resources for research and breeding
- Genomic studies are in progress

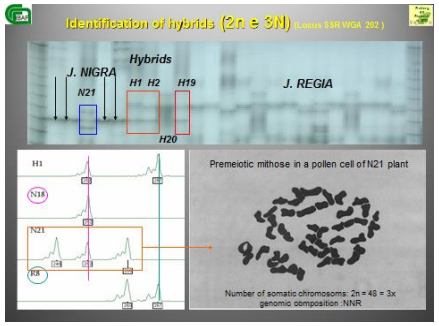
Infestation potential of *Dryocosmus kuriphilus* Yasumatsu, 1951 (Hymenoptera: Cynipidae) in different natural populations of *Castanea sativa* Miller: an experimental ex-situ test
Bombi, Fedi, Zapparoli, Cammarano, Guidolotti, Pallozzi, Gaudet, Mattioni, Cherubini, Beritognolo, Villani

International Journal of Pest Management. Accepted for publication

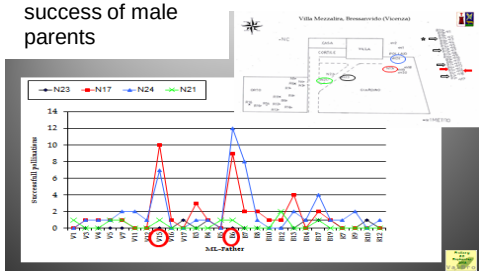
Genetic improvement for interspecific walnut hybrids
(cooperation with CREA)

Juglans regia

Selection of hybrid walnut plants in common garden. Identification of maternal hybridogenic trees, putative fathers, and hybrid progenies.

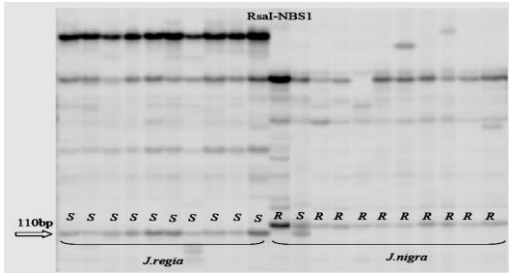


Reproductive success of male parents



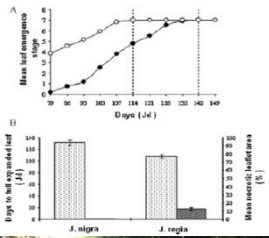
Selection of *J. nigra* and *J. regia* genotypes susceptible/tolerant to biotic stress (Gnomonia leptostila) by neutral and functional markers

Juglandetum CREA



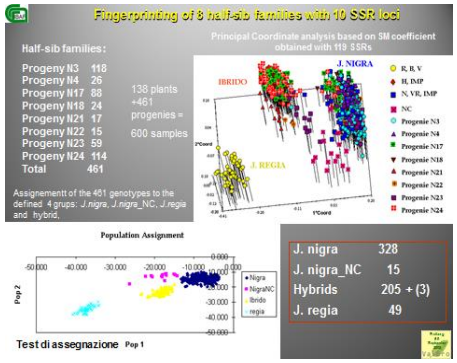
Functional molecular markers associated with Resistant or Resistant-analogs genes used for the early selection of genotypes tolerant/susceptible to diseases. (NBS profiling approach)

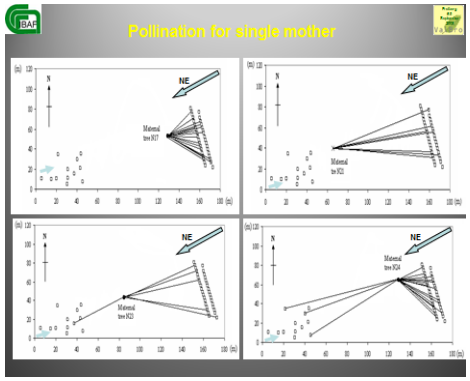
Avoidance by late flushing



Pathogenicity test in vivo

Controlled interspecific crosses





Mol Breeding (2009) 24:321–335
DOI 10.1007/s11032-009-9294-7

Retrospective identification of hybridogenic walnut plants by SSR fingerprinting and parentage analysis

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Mechanisms governing the responses to anthracnose pathogen in *Juglans* spp.

P. Pollegioni[a,*], G. Van der Linden[b], A. Belisario[c], M. Gras[d], N. Anselmi[e], I. Olimpieri[a], L. Luongo[c], A. Santini[g], F. Turco[g], G. Scarascia Mugnozza[f], M.F. Malvolti[a]

Juglans regia

Phenotypic characterization of productive traits (fruit quality) in walnut

Improvement of local varieties and ecotypes: molecular, morphological, biochemical markers

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Juglans regia Provenance Research by Molecular, Morphological and Biochemical Markers: A Case Study in Italy

Maria Emila Malvolti[1] • Paola Pollegioni[1] • Alcide Bertani[2] • Sergio Mapelli[2]*

Ecotipi/varietà locali poco valorizzati e a rischio estinzione

Pericolo di erosione genetica

Caratteristiche di rusticità, adatto per riforestazione e sistemazione idrogeologica

Mancanza di strategie di valorizzazione

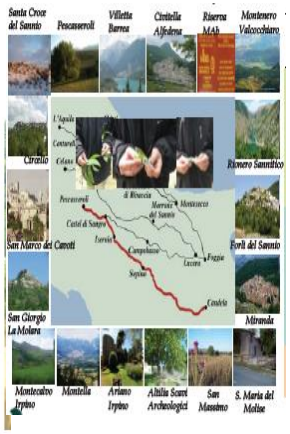
Produzioni e superfici in continuo calo

1) Individuazione di una macroarea-studio

2) Analisi della nocicoltura di montagna

3) Uso di metodologie analitiche per il controllo e la caratterizzazione

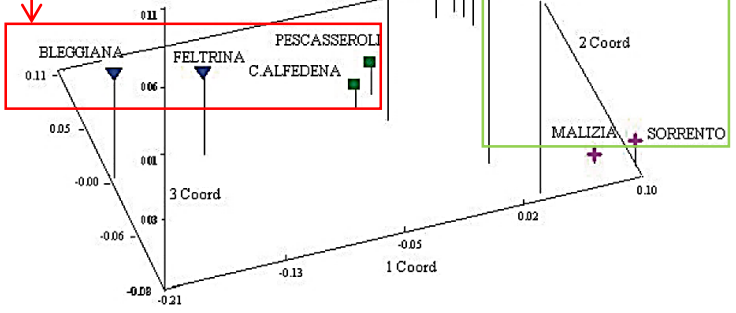
4) Costruzione di strategie territoriali per la valorizzazione



Alt. mt 700/1200 mt, clima rigido

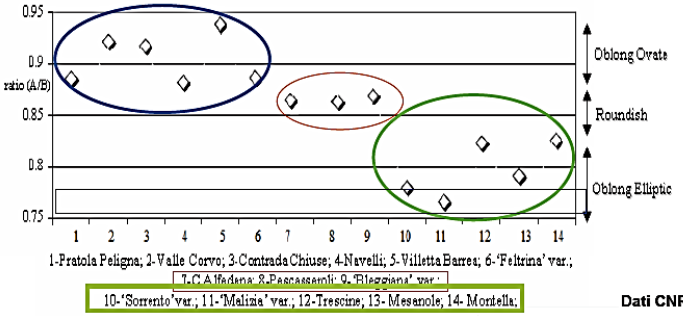
Alt.700 mt, clima fresco - temperato

Alt. 300 mt, clima caldo-temperato



Analisi delle coordinate principali basata su 10 SSR

Varietà conosciute di confronto:
Sorrento e Malizia (Campania), Bleggiana (Trentino) e Feltrina (Veneto)

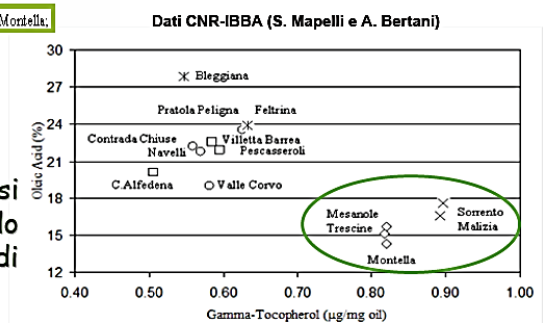


RISULTATI

Forma frutto: rapporto diametro Equatoriale / Polare

Regione Campania
Regione Abruzzo
Regione Molise

Tocoferolo, acidi grassi poliinsaturi (ω3; ω6) ed acido oleico contenuti nei frutti di noce.



Towards more collaborative *multidisciplinary research activities for* tree breeding in Italy?

- **Agro-forestry species are multipurpose and ecologically relevant, but with low economic impact**
- **Tree species require permanent infrastructures for long-term research and breeding**
- **Characterised tree genetic resources available in collections are valuable for breeding but underexploited**
- **Part of the novel scientific knowledge not yet used in innovative Italian tree breeding programmes**
- **How to improve the system?**

Thanks for your attention!