Fingerprinting of Sicilian cherry germplasm with Simple Sequence Repeats and incompatibility (*S*) locus primers

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a) Sicilian sour cherry, domesticated from the wild

Introduction

Sicily has distinctive germplasm of diploid sweet cherry (*P. avium*) and tetraploid sour cherry (*P. cerasus*), cultivars as well as wild and/or escaped to the wild genotypes. Cherries are generally cultivated in the traditional way – often as isolated trees or in mixed orchards. They are also a frequent component of woodland and road margins. Many cultivars are disappearing as farmers stop growing them. The Department of Colture Arboree of Palermo University has started a programme to evaluate and conserve cherry genetic resources.

This study is assessing the genetic diversity of Sicilian cherry accessions and determining their self-incompatibility genotypes, using a novel method developed at East Malling Research UK, based on multiplexing a set of Simple Sequence Repeats primers with incompatibility (*S*) locus primers (Vaughan & Russell, 2004, Vaughan et al. submitted).

b) Sweet cherry 'Sampitrisa' tree; c) 'Cappuccia Bivona' fruits

Materials and Methods

- 40 accessions of sweet cherry representing the majority of Sicilian cultivars, one indigenous wild sweet cherry and the international cultivar 'Napoleon'.
- 25 accessions of sour cherry from the wild and farms (Fig. 1).
- Genomic DNA extracted from buds according to Doyle and Doyle (1997).
- 10 fluorescently labelled SSR primer pairs multiplexed in two PCRs – EMPaS: 02, 06, 11, 14; EMPa: 04, 05, 15, 18 (East Malling); UDP98-412 (Udine); and PceGA34 (Michigan) – along with two primer pairs for the incompatibility (*S*) locus, amplifying across the first intron of the *S-RNase* gene and across the intron of the *SFB* gene.
- PCR product were sized using an ABI Prism 3100 running GENESCAN® and GENOTYPER® software
- A UPGMA dendrogram was constructed using PAUP*.

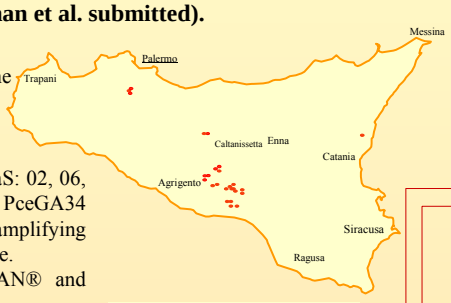

Fig. 1. Map of Sicily showing the location of the sour cherry genotypes studied

Results

Sweet

- The number of alleles per SSR locus ranged from 4 to 11. The SSRs distinguished 33 Sicilian accessions; eight accessions fell into four non-distinguishable pairs, indicating synonymy. Some accessions with same name but of different origin were distinguished (Fig. 2).
- 'Napoleon' did not separate from Sicilian accessions, but it was distinct from the 'Napoleona' accession groups from Sicily. The wild accession 'Ciliegio Selvatico' did not separate from the cultivars.
- Accessions were assigned to different incompatibility groups (Table 1). The most frequent groups were *S3S6*, *S3S9*, *S3S16* and *S6S16*.
- Alleles *S16* and *S22* were frequent, though they generally occur in wild populations (De Cuyper et al. 2005), suggesting that some accessions may derive from cross-pollination between cultivated cherries and wild cherries (Fig. 3)

TAB. 1. INCOMPATIBILITY GENOTYPES IN SICILIAN SWEET CHERRIES

Accession	Incompatibility Genotype	Group*
Zu Pippinu	*S1S3*	II
Quarantana/Virgulisa	*S2S3*	IV
Cappuccia Castelbuono Giniusa Maiolina Messina Napoleona Precoce Toscana/Toscanella Muddisa	*S3S6*	VI
Maiolina Rappu	*S3S5*	VII
Maiolina Etna Napoleona/Napoleona Virifica	*S6S9*	X
Sampitrisa B	*S6S13*	XII
Dura	*S1S5*	XIV
†Kronio	*S5S6*‡	XV
Cristallina/Moretta Furistera Napoleona Maiolina Castelbuono Napoleona Tardiva Napoletana Castelbuono	*S3S9*	XVI
Durona Minnulara Napoletana Sampitrisa A	*S3S16*	XXIII
Cappuccia Bivona Cappuccia Doppia	*S6S22*	New
Caddusa Cappuccia Chiusa Sclafani	*S13S22*	New
Cappuccia Imperiale Forma di Cuore	*S6S16*	New
Carrubbedda Niura dell'Etna	*S3S22*	New
Cavallaro	*S5S9*	O
Ciliegio Selvatico	*S21S22*	O
Don Antonio	*S16S22*	O
Moscatella	*S17S21*	O
Nucigliera	*S14S16*	O

/ Possible synonyms
* According to Tobutt et al. 2004
† Reportedly self-compatible (Calabrese et al. 1984)
‡ Data to be confirmed

Sour

- The number of alleles per SSR locus ranged from 4 to 9.
- A low level of diversity was found among the 25 wild genotypes suggesting they may be derived from closely related seedlings or suckers.
- 24 genotypes showed the same incompatibility phenotype *S6*, *S13*, *SB*, *SD* (according to the allele nomenclature adopted by Tobutt et al. 2004).
- One genotype was *S10*, *S16*, *SB*, *SD*, indicating it may be a Duke cherry.

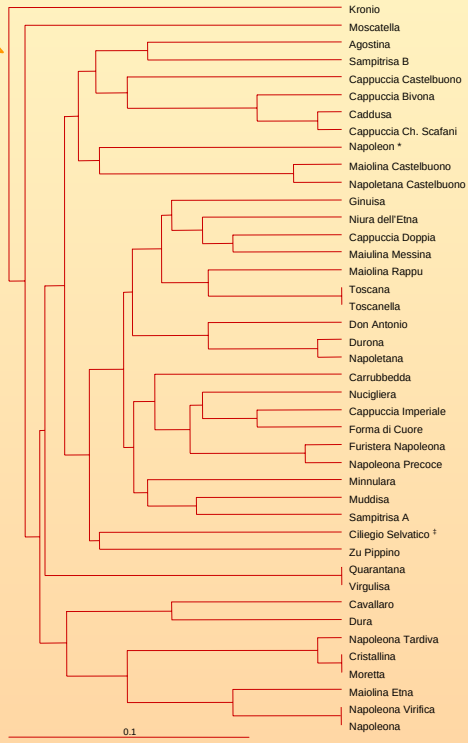

Fig. 2. UPGMA dendrogram showing genetic relationship among 41 Sicilian sweet cherries, based on 10 SSR loci and the *S*-locus. *International. ‡ Wild

Conclusion & Outlook

- The Multiplex method was useful for the analysis of incompatibility genotypes and molecular characterisation and could be useful for a world-wide comparison of cherry germplasm.
- Knowledge of the incompatibility genotypes could help farmers to choose appropriate pollinators to maximize production and breeders to design specific crosses.
- A larger number of sour cherry genotypes from across Sicily will be studied.

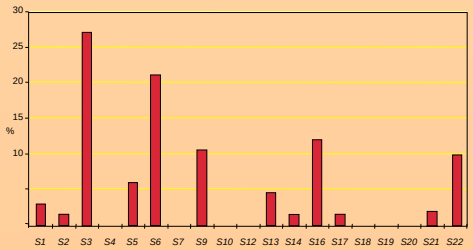

Fig. 3. Occurrence of *S*-alleles in the Sicilian sweet cherries

- Test crossing and analysis of stylar ribonucleases will be undertaken to study self-compatibility in the cultivar 'Kronio'.
- This work could contribute to a database for breeding and conservation purposes.

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