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# Genetic diversity of fig (*Ficus carica* L.) genotypes grown in Southern Italy revealed by the use of SSR markers

F. Costa<sup>1</sup>, A. Marchese<sup>1</sup>, R. Mafrica<sup>2</sup>, C. Di Vaio<sup>3</sup>, G. Ferrara<sup>4</sup>, S. Fretto<sup>1</sup>, A. Quartararo<sup>1</sup>, F.P. Marra<sup>1</sup>, C. Mennone<sup>5</sup>, F. Vitale<sup>6</sup>, S. Reale<sup>6</sup>, T. Caruso<sup>1</sup>

<sup>1</sup>Dipartimento Scienze Agrarie e Forestali, University of Palermo, Italy;

<sup>2</sup>Dipartimento di Agraria, University Mediterranea of Reggio Calabria, Italy;

<sup>3</sup>Dipartimento di Agraria, University Federico II, Naples, Italy;

<sup>4</sup>Dipartimento di Scienze del suolo, della pianta e degli alimenti, University of Bari, Italy;

<sup>5</sup>Experimental farm Pantanello Alsia, Region Basilicata, Italy;

<sup>6</sup>Istituto Zooprofilattico Sperimentale della Sicilia, Palermo, Italy.

## Abstract

The genetic variability among 181 fig (*Ficus carica* L.) accessions found in small farms located in Campania, Basilicata, Apulia, Calabria and Sicily was investigated analysing the polymorphism of 18 simple sequence repeat (SSR) markers. The SSR analysis revealed a large genetic diversity among accessions. A total of 117 alleles were detected with a mean of 6.5 locus-1. The average expected ( $H_e$ ) and observed heterozygosity ( $H_o$ ) were 0.56 and 0.66, respectively. The mean polymorphic information content (PIC) was 0.51, suggesting a significant molecular diversity among the fig accessions taken into consideration. The UPGMA cluster analysis discriminated 174 genotypes and allowed to find 8 groups of identity. The genotype 'Bianca di agosto' was an "outgroup". This study showed groups of fig accessions matching with their geographic or cultivation area, but also many genotypes, especially from Campania, with uneven distribution. The research evidenced the richness of the available fig genetic resources in Southern Italy, resolved cases of synonymies and homonymies and helped to characterize fig accessions, an important preliminary work for establishing core collections.

**Keywords:** germplasm, genetic resources, genetic relationships, microsatellites

## INTRODUCTION

In Italy, the common fig (*Ficus carica* L.) is one of the oldest cultivated fruit tree, commonly present in family orchards. The ancient traditions of fig cultivation, the easy dissemination of seeds by birds, humans and other animals, the strong internal variability of the species have facilitated not only the spread of the species but also the emergence of different forms that farmers have selected and from which cultivars originated. In the early XIX century, Galesio described numerous cultivars in his monumental work "Italian Pomona" (Galesio, 1820) and, for some of them reported some wonderful botanical illustrations. Afterward several authors have dealt with the Italian varietal heritage of this species (De Rosa, 1901; Vallese, 1909; Fratapietro, 1930; Donno, 1948, 1951a,b, 1952, 1959; Condit, 1955; Pasquini, 1956; Milella, 1958; Basso 1960a, b; Casini et al., 1990; Chessa and Nieddu, 1990; Ferrara, 1990; Ferrara and Vendola, 1990; Grassi et al., 1990; Grassi, 1997). Although there are many descriptions of the fig cultivars grown in Italy, they are incomplete, lacking standardised method of characterisation. In most cases, cultivars were described in their area of cultivation and

therefore in very different environments, which affected biometric and morphological characters, making difficult the comparison between genotypes, especially among those that exhibit a high degree similarity. Thus, there is the need to make order and clarity on the fig genetic resources present in Southern Italy.

Since morphological and agronomic characters are affected by environmental conditions and they can vary depending on the complex floral biology of fig, molecular markers are used as complementary tool to assess the genetic diversity. In fig, many genetic study have been conducted with various molecular methodologies, such as isozymes (e.g., Chessa et al., 1998), RAPDs (e.g., Khadari et al., 1995) , AFLPs (Cabrita et al., 2001) and SSRs (e.g., Khadari et al., 2004; Giraldo et al., 2005, 2008). In 2014, the Universities of Palermo, Reggio Calabria, Napoli and Bari and the Agricultural office of the region Basilicata started a collaborative programme and conducted an extensive investigation in traditional fig growing areas to identify different fig cultivars and genotypes, in order to collect new knowledge on fig genetic resources in Southern Italy. These cultivars and genotypes were characterized with microsatellite markers for the first time and preliminary results are reported here.

## **MATERIALS AND METHODS**

Young leaves were collected from 181 traditional figs cultivars and genotypes, cultivated in five Italian regions of South Italy: Apulia, Basilicata, Calabria, Campania and Sicily. Samples were frozen in nitrogen liquid until use. Genomic DNA was extracted following the method of Doyle and Doyle (1987) with modifications. Eighteen microsatellite markers were employed for the molecular characterization (Table 1). PCR amplicons were analysed with the Applied Biosystems 3130 genetic analyser capillary sequencer and they were sized using GeneScan Analysis software. Number of alleles/locus (No), observed and expected heterozygosity ( $H_o$  and  $H_e$ ) and polymorphic information content (PIC) values were calculated with Power Markers v.3.25 (Liu and Muse, 2005) (Table 1). A UPGMA dendrogram was constructed, using the coefficient of similarity of Nei (1973) with the software Power Markers v.3.25, in order to illustrate the genetic diversity among genotypes (data not shown). To evaluate the genetic structure of fig germplasm, the software Structure (Pritchard et al., 2000; Falush et al., 2003, 2007) was employed, using the 'admixture' model, assuming 1-10 populations (K), a burn-in length of 10,000, followed by 90,000 runs at each K, with 10 replicates for each K. The log likelihood for each K ( $L(K)$ ) was used (Rosenberg et al., 2002) to determine the right number of populations (K). Genotypes having membership probabilities  $\geq 0.80$  were considered to belong to the same group, sharing a close genetic background.

## **RESULTS AND DISCUSSION**

The SSR were polymorphic and revealed a large genetic diversity among the accessions. A total of 117 alleles were detected with a mean of 6.5 locus-1 (Table 1). The average expected ( $H_e$ ) and observed heterozygosity ( $H_o$ ) were 0.56 and 0.66, respectively. The mean polymorphic information content (PIC) was 0.51, indicating a high molecular diversity among the fig accessions of the Southern Italy germplasm. The UPGMA cluster analysis allowed to discriminate 174 genotypes and to identify eight groups of identity (data not shown). The accession 'Bianca d'Agosto' from Basilicata and the genotype "CS138" from Calabria were the most diverse. This study showed that in some cases fig accession grouping followed the area of cultivation, but also many genotypes, especially from Campania, presented uneven distribution (data not shown). In the structure analysis, fig genotypes were grouped in four populations (Figure 1). The 'Dottato' genotypes belonged to the yellow population, while most

of the 'Melanzana type' figs were included in the black population. The name 'Melanzana' indicates the resemblance of the colour of these figs to that of an eggplant. The black population contained also the cultivar 'Paradiso'. The green population contained some late accessions such as 'Nvernitica', 'Natalina', 'Regina' and 'Troiano'. The red population was quite heterogeneous, as regards the phenology (ripening time), the type of fruit (brebas or figs) and the characteristics of the fruit. It included accessions producing big and good quality brebas ('Fiorone San Giovanni', 'Fiorone Sant'Antonio') and other accessions that need to be further characterised. Many accessions presented mixed genetic background. Since this is a preliminary work and considering that the morphological characterisation is still in progress, in some cases the interpretation of results was difficult. The research evidenced the richness of the available genetic resources in Southern Italy, resolved cases of synonymies and homonymies and helped to characterize fig accessions, an important preliminary work for establishing core collections or for planning on-farm conservation measures. In the near future, the molecular characterisation will be complemented with the morphological characterisation of all accessions and thus we will have complete information on Italian fig diversity.

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Table 1. SSR statistics: loci names, number of alleles (No), references, values of observed ( $H_o$ ) and expected ( $H_e$ ) heterozygosities and polymorphic information content (PIC).

Locus	Reference	Allele No	$H_e$	$H_o$	PIC
FCUP027_4	Bandelj et al. (2007)	6.00	0.68	0.87	0.63
FCUP038_6	Bandelj et al. (2007)	13.00	0.72	0.81	0.69
FM4_70	Zavodna et al. (2005)	8.00	0.50	0.43	0.46
LMFC12	Giraldo et al. (2005)	6.00	0.53	0.70	0.44
LMFC13	Giraldo et al. (2005)	5.00	0.48	0.58	0.42
LMFC19	Giraldo et al. (2005)	7.00	0.40	0.37	0.38
LMFC24	Giraldo et al. (2005)	4.00	0.36	0.41	0.31
LMFC26	Giraldo et al. (2005)	4.00	0.22	0.24	0.21
LMFC28	Giraldo et al. (2005)	6.00	0.64	0.63	0.58
LMFC30	Giraldo et al. (2005)	9.00	0.80	0.90	0.78
LMFC32	Giraldo et al. (2005)	5.00	0.37	0.20	0.34
MFC1	Khadari et al. (2001)	6.00	0.67	0.99	0.61
MFC2	Khadari et al. (2001)	8.00	0.67	0.91	0.61
MFC3	Khadari et al. (2001)	10.00	0.67	0.79	0.62
MFC7	Khadari et al. (2001)	4.00	0.49	0.76	0.38
MFC8	Khadari et al. (2001)	5.00	0.61	1.00	0.54
MFC9	Khadari et al. (unpublished)	5.00	0.51	0.36	0.44
MFC11	Khadari et al. (unpublished)	6.00	0.68	0.97	0.64
Mean		6.44	0.61	0.66	0.51

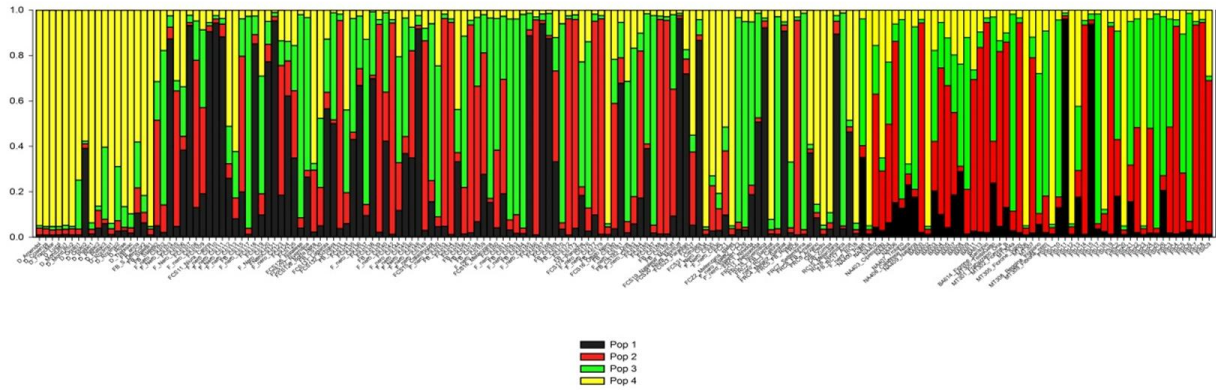


Figure 1. Inferred population structure for  $K=4$  of 181 fig accessions from Southern Italy.

