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Short communication

The origin of the self-compatible almond 'Guara'

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ABSTRACT

'Guara' has been the most planted almond cultivar in Spain in recent years. The introducers of this cultivar reported its origin as unknown and suggested that it is related to the Italian almond cultivar 'Tuono'. Indeed, the experience of farmers and researchers has revealed strong similarities between 'Guara' and 'Tuono'. In order to compare the identity of the two cultivars, their genetic profiles (fingerprints) were determined with a set of 12 SSR markers used to analyse the INRA clones of 'Guara' and 'Tuono' as well as two clones considered synonymous with 'Tuono', namely 'Supernova' and 'Mazzetto'. A supplementary set of 23 SSRs was also analysed in 'Guara' and 'Tuono' samples from different reference collections of CEBAS-CSIC (Murcia, Spain), INRA (Avignon, France), IRTA (Constantí, Spain), and the University of Bari (Bari, Italy). The results confirmed that 'Guara' and 'Tuono' present identical DNA fingerprints for the 35 SSRs analysed. This genotypic information together with the similar characteristics of the two cultivars demonstrates that Spanish 'Guara' is actually the same cultivar as the Italian 'Tuono'.

1. Introduction

The almond (*Prunus dulcis* Mill.) 'Guara' has been the most widely planted cultivar in Spain in recent years (1996–2010), representing almost 40% of all new plantations (Socias i Company et al., 2009, 2011, 2012; Socias i Company and Couceiro, 2014). Its extensive use can be attributed to its agronomic performance, given that it was the first self-compatible late flowering cultivar available in the country. In addition, government support stimulated uptake of this variety, despite its intermediate vigour and productivity, slightly bitter kernel flavour, susceptibility to various fungus diseases and the presence of double kernels. Nevertheless, due to its consistency in production and resistance to frost it is still planted in Spain (Socias i Company et al., 2011, 2012; Socias i Company and Couceiro, 2014).

'Guara' was named in 1987 in the "Unidad de Fruticultura" of CITA in Zaragoza (Spain) (Felipe and Socias i Company, 1987; Kester, 1994). According to its introducers, 'Guara' is of unknown origin and it came from a clonal and sanitary selection of a mislabelled accession found in the CITA almond collection in 1974. In the CITA breeding programme, besides 'Guara', eight other almonds have been released ('Aylés', 'Moncayo', 'Blanquerna', 'Cambra', 'Felisia', 'Soleta', 'Belona' and 'Mardía'). However, according to information provided by the breeders, of the nine releases, 'Guara' represented around of 95% of CITA cultivar plants propagated in nurseries between 1996 and 2010 (Socias i Company et al., 2011, 2012; Socias i Company and Couceiro, 2014).

'Guara' was registered in the "Spanish Register of Protected Varieties" in 2003, which would have protected the variety for a period of 20 years (Boletín Oficial del Estado Español, 2003), but the protection was cancelled in 2012 (Boletín Oficial del Estado Español, 2012), despite the wide dissemination and multiplication of the cultivar in Spain during the nine years it was registered (Socias i Company et al., 2012; Socias i Company and Couceiro, 2014).

For a long time, research workers, technicians and farmers have observed similar traits and agronomic behaviour in 'Guara' and the Italian cultivar 'Tuono' (flowering time, self-compatibility, productivity, ripening time, vigour of tree, fruit shape, fruit flavour and fungus disease susceptibility) (Grasselly and Duval 1987; Navarro 2002; Muncharaz 2004; Arquero et al., 2008; Arquero 2013). For this reason, together with the unknown origin of the cultivar, dif-

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ferent researchers have suspected that 'Guara' might in fact be the native Italian 'Tuono'.

'Tuono' is one of the most remarkable local Italian varieties. It first spread in Apulia around 1830, in the territory of Trinitapoli (Foggia province), where it was the only cultivar grown (Pantanelli and Fanelli, 1934). Its first name 'Mandorla del Trono' ("trono" in Italian means throne and was subsequently modified to 'Truono' or 'Tuono') was probably linked to the time when it was introduced, during the period of Bourbon rule (Fanelli, 1939). After 100 years, 'Tuono' had spread to other Apulian areas, in some of which it is also known by the names of 'Troito' or 'Troilo' (Fanelli, 1939). In 1970, this cultivar represented 10–40% of the plantations in these areas, depending on location (Grasselly and Crossa-Raynaud, 1980). It was later introduced to Greece where it is known as 'Truoito' (Stylianides, 1976) and to Libya and Tunisia where it is known as 'Mazzetto' (Grasselly and Olivier, 1976). At present, most of the new almond orchards in southern Italy are planted with 'Tuono' due to its late blooming, self-fertility and wide environmental adaptability (Sottile et al., 2014). Interestingly, the cultivar 'Supernova', released as a self-compatible mutant of 'Fascionello', was found to be 'Tuono' (Marchese et al., 2008).

Since the first report of self-compatibility of certain Apulian varieties (Grasselly and Olivier, 1976), including 'Filippo Ceo' first and then 'Tuono', this cultivar has long been used as a source of self-compatibility and late flowering in different European breeding programmes. These include those at INRA Avignon, France where is the progenitor of 'Lauranne', 'Steliette' and 'Mandaline'), CITA Zaragoza, Spain ('Aylés', 'Moncayo', 'Cambra' and 'Felisia'), CEBAS-CSIC Murcia, Spain ('Antoñeta' and 'Marta'), and IRTA Constantí, Spain ('Francolí').

Today, molecular markers such as SSRs (Simple Sequence Repeats) make it easy and economical to identify cultivars of almond. In this species, a set of five selected SSRs have made it possible to identify most almond varieties, even those coming from the same cross [(such as 'Marta', 'Antoñeta' and 'Lauranne', descendants of 'Ferragnès' × 'Tuono') (Sánchez-Pérez et al., 2006)]. In addition, for the identification of the Californian almond varieties, Dangl et al. (2009) used 12 SSRs to identify 18 almond cultivars.

Different SSR markers have been applied to the identification of 'Guara' and 'Tuono' by different groups who were unable to differentiate between these cultivars (Sánchez-Pérez et al., 2006Gouta et al., 2010). Only the team from CITA Zaragoza reported that these varieties were similar but slightly different with 19 SSR markers and suggested that 'Guara' could have originated by self-pollination of 'Tuono' (Fernández i Martí et al., 2009).

The objective of this study was the molecular characterization of accessions of 'Guara' and 'Tuono' cultivars from four different germplasm collections in France, Spain and Italy based on an analysis of 35 SSRs, including those analysed by Fernández i Martí et al. (2009), in independent laboratories. The goal of this characterization is to determine if 'Guara' and 'Tuono' are the same or different cultivars

2. Materials and methods

Leaf samples of 'Guara' and 'Tuono' (together with 'Mazetto' and 'Supernova', synonyms of 'Tuono') were picked from the INRA Avignon collection for the analysis of 12 SSRs in the Avignon laboratory (Table 1). In addition, leaves were collected from the official germplasm banks of CEBAS-CSIC (Murcia, Spain), INRA (Avignon, France), IRTA (Constantí, Spain), and the University of Bari (Bari, Italy). Leaf samples, identified with a code, were sent to the Genetic Analysis Service of CRAG (Barcelona, Spain) for analysis with a set of 23 SSRs (Table 2).

Table 1
Size of alleles obtained in the analysis of 12 SSRs in samples of the almond 'Guara', 'Tuono', 'Mazzetto' and 'Supernova' from INRA Avignon. For the four cultivars, the alleles of all SSRs were identical.

SSR markers	Reference	Alleles size
BPPCT010	Dirlewanger et al. (2002)	139/160
BPPCT027	Dirlewanger et al. (2002)	241/249
BPPCT036	Dirlewanger et al. (2002)	248/250
CPDCT005	Mnejja et al. (2004)	101
CPDCT034	Mnejja et al. (2004)	172/173
CPDCT035	Mnejja et al. (2004)	142/154
pchgms1	Sosinski et al. (2000)	184/203
pchgms3	Sosinski et al. (2000)	173/175
UDP96-003	Cipriani et al. (1999)	100/104
UDP96-013	Cipriani et al. (1999)	140
UDP96-018	Cipriani et al. (1999)	231/235
UDP98-408	Cipriani et al. (1999)	101

Table 2
Allele sizes of 23 SSRs obtained in the analysis of samples of almonds 'Tuono' and 'Guara' from CEBAS-CSIC, INRA, IRTA and the University of Bari. The allele size for all SSRs was identical for 'Tuono' and 'Guara' from the four germplasm collections. NA = no amplification.

SSR markers	Reference	Allele size
BPPCT001[a]	Dirlewanger et al. (2002)	151/160
BPPCT007[a]	Dirlewanger et al. (2002)	126/150
BPPCT018[a]	Dirlewanger et al. (2002)	NA
BPPCT025[a]	Dirlewanger et al. (2002)	170/180
BPPCT033	Dirlewanger et al. (2002)	122/145
BPPCT038[a]	Dirlewanger et al. (2002)	124/151
CPDCT025[a]	Mnejja et al. (2004)	199/201
CPDCT045[a]	Mnejja et al. (2004)	138/164
CPPCT006[a]	Aranzana et al. (2002)	180/186
CPPCT022[a]	Aranzana et al. (2002)	227
CPPCT033[a]	Aranzana et al. (2002)	135/153
CPPCT044[a]	Aranzana et al. (2002)	166/192
CPSCT012[a]	Mnejja et al. (2004)	151/157
CPSCT018[a]	Mnejja et al. (2004)	159
CPSCT021[a]	Mnejja et al. (2004)	151/157
EPDCU3083[a]	Howad et al. (2005)	133
EPDCU5100[a]	Howad et al. (2005)	172/175
EPPCU0532	Howad et al. (2005)	172/174
EPPCU6216	Howad et al. (2005)	168/195
EPPCU9168[a]	Howad et al. (2005)	180/192
PGS12e2	Soriano et al. (2012)	154/158
PMS40[a]	Cantini et al. (2001)	111/117
UDP96-005[a]	Cipriani et al. (1999)	137/156

[a] SSRs assayed by Fernández i Martí et al. (2009).

SSR analysis: In both laboratories, INRA and CRAG, DNA was extracted using the CTAB method according to Doyle and Doyle (1987) but scaled down to a 1.5 ml tube format. A total of 35 SSRs were analysed (Tables 1 and 2), including all the SSRs assayed by Fernández i Martí et al. (2009), which are indicated in Table 2 with asterisks. DNA fragment separation was performed in an ABI Prism 3130*xl* automated sequencer (PE/Applied Biosystems).

3. Results

Table 1 shows the allele sizes of 12 SSR markers for 'Guara', 'Tuono', 'Supernova' and 'Mazetto' from the INRA collection. All cultivars presented identical fingerprints for all of the 12 SSRs used.

Table 2 shows the size of the SSR alleles of the 23 markers assayed in the analysis of the 'Tuono' and 'Guara' samples from the different germplasm banks of CEBAS-CSIC, INRA, IRTA and the University of Bari. 'Guara' and 'Tuono' presented identical DNA fingerprints for all 23 SSRs.