DISCRIMINATION AND ASSISTED SELECTION OF "SAN MARZANO" BY MEAN OF A NEW MOLECULAR MARKER FOR FRUIT SHAPE

N. ACCIARRI*, E. SABATINI*, S. VOLTATTORNI*,T.CIRIACI*, G. L. ROTINO**

***) C.R.A. Istituto Sperimentale per l'Orticoltura, Via Salaria 1, 63030 Monsampolo del Tronto (AP)**
****) C.R.A. Istituto Sperimentale per l'Orticoltura, Via Paullese 28, 26836 Montanaso Lombardo (LO)**

Introduction

The typical and famous peeled tomato grown for processing in Italy and particularly in the Campania region is the San Marzano variety. This traditional variety, grown for fresh market too, is protected since 1999 by a European Union DOP (Protected Denomination of Origin) certification as "Pomodoro San Marzano Dell'Agro Sarnese-Nocerino" (Figure 1).
S. Marzano production in the Campania region has declined significantly in the last decades due to high sensibility to vascular diseases and cucumber mosaic virus, together with economical issues arisen for the low resistance to mechanical processing and to the lower yield than the modern F1 hybrids. In the 1980s, this region was the number one for peeled tomatoes production in Italy. Now, Campania is the 4th or 5th produced with 35% of the peeled tomatoes grown in Italy. Production of typical S.Marzano variety in Campania is declining at a rate of about 12-16% a year. The concern is that the San Marzano tomato will disappear from the Campania region.
Therefore, for processing and for fresh market consumption, new F1 hybrids, with similar shape, have been preferred to the original San Marzano; these hybrids have a different taste and are often genetically far from San Marzano, but possess genetic resistance to the most threatening tomato pathogens.
Some genetic tools have already been developed to characterize the original accessions. These method are mainly based on the characterization of hyper variable regions of the genome (Rao *et al*, 2006), but are less used in marker assisted selection for the introgression of genetic resistances, where a fast and high-throughput method is required.

We developed a CAPS marker for the "ovate" gene in tomato (Sabatini et al, 2005). It was developed to assist selection when genetic resistances or other features are introgressed from elongated or round-fruited cultivar to neck-constricted ones.
Here we report the finding that the original accessions of San Marzano carry the *ovate* gene in its mutated form, which confer neck constriction, while the most modern F1 hybrids, resembling San Marzano, do not.

Materials and Methods
In order to analyze fruit morphology of different lines and cultivar correctly or incorrectly labeled as "San Marzano", 27 tomato accessions were analyzed for fruit shape features as described by van der Knaap and Tanksley, 2003. As reported in figure 2, fuit shape features, such as neck constriction, fruit weight and an empiric index of "pear" shape, were measured. The 27 accessions included some "San Marzano" original lines from Campania (10 accessions), F1 hybrids with elongated fruit for fresh market (5 accessions), F1 hybrids for processing (9 accessions), Italian typical cultivars (2 accessions) and the F1 resulted crossing a "San Marzano" with a round fuited tomato (called "tondo").

Results
Fruit shape variability resulted very high despite the presence or not of the *ovate* alleles, as shown in the figures 2 and 6, confirming the fact that *ovate* locus probably interact with other, in part still unknow, loci in the genome (Tanksley, 2004)
As shown in figure 3 and 4, the accessions used for morphological studies were analyzed with the CAPS marker developed by Sabatini et al (2006). "Yellow pear" and "Mogeor" were used as tests for both the homozygous conditions; "San Marzano x Tondo" was used to test the efficacy of the marker on heterozygous material. Surprisingly, only the San Marzano original lines own the ovate mutated gene in homozygous condition; only N.S.Roma is homozygous for ovate but it cannot be included in San Marzano group. HF1 Galeon and Italdor shows both wild type and mutated alleles indicating that only one of the parents were ovate/ovate. As shown in figure xxx, even if Galeon possess a quite pronounced neck constriction (confirming the incomplete dominance of ovate), it doesn't match the distinctive features of the berry of San Marzano UPOV descriptors.

Discussion
The CAPS marker developed by Sabatini et al (2006) was already successfully employed in marker assisted selection for the introgression of genetic resistances in "Cuor di Bue di Albenga" lines, when a round type, multi-resistant cultivar was used as donor parent.
In our experiments, only the 11 San Marzano original lines resulted homozygous for ovate. Therefore, it can be assumed that San Marzano lines are homozygous for ovate gene in its mutated form, but of course it cannot be stated that ovate gene identifies exclusively San Marzano tomatoes.
Therefore this genetic tool can be useful for a first, fast and cheap screening for the identification of original San Marzano accessions and, together with the already developed (GATA)4 fingerprinting (Rao et al, 2006), is an interesting tool to solve distinction problems for the San Marzano tomato toward not original foodstuff, yet ambiguously labeled as "San Marzano".
Furthermore, this marker can be usefully employed for genetic improvement of the original San Marzano for the introgression of genetic resistances when a round or elongated "not-ovate" line is used as source of resistances.

Figure 1: geographic location in Campania region of the areas included in the certification of the European DOP for the cultivation of the "San Marzano dell'Agro Sarnese-Nocerino

Figure 2: fruit shape analysis of some original San Marzano accession compared with the most diffuse S.Marzano kind-F1 hybrids and other F1 mainly used for processing. Notice that some of the elongated F1 hybrids shows indeterminate growth and are grown also for fresh market purposes. Pear shape index: empiric pear shape index, 1=round, 3 = pear shape (e.g. Yellow Pear=3); sblk= stem blockiness, x=diameter at 10% of total fruit length from the proximal end of the fruit, y= max diameter

Figure 3: 1:Yellow Pear (*ovate/ovate*) ; 2:Mogeor (round, +/+) ; 3: Marzano; 4: Campano; 5: S.Marzano Baldoni ; 6: S.Marzano 622; 7: HF1 S.Marzano x Tondo; 8: S.Marzano L6; 9: S.Marzano L8; 10: S.Marzano 618; 11: S.Marzano 619; 12: UC 82; 13: N.S.Roma; 14: Malareto; 15: Galeon; 16: Perfect Peel; 17: Nocerino.

Figure 4: 1:Yellow Pear; 2:Mogeor; 3: Marzano; 4: Campano; 5: Allflesh 915 ; 6: Coimbra; 7: Alfa 200; 8: Castelong; 9: Red Peel; 10: PS 38591; 11: CLX 37203; 12: CLX 37201; 13: CLX 37198; 14: Oskar; 15: Italdor; 16: Corianne DR.

Figure 5: Galeon F1 (left) vs S.Marzano: notice the neck constriction of Galeon (ovate/+) similar to the S.Marzano one. However, Galeon unripe fruit shows a pale green epidermis and does not present green shoulder, therefore it does not not match the parameters indicated by UPOV for identification of "San Marzano"

Figure 6: pronounced neck constriction in an elongated "Red Peel" fruit (left), compared with a San Marzano line. Red Peel resulted omozygous for wild-type alleles at the *ovate* locus (e.g.as in round-fruited genotype); its particular shape could be probably attributed to the effect of "*sun*" locus in this genetic background.

References

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Figure1

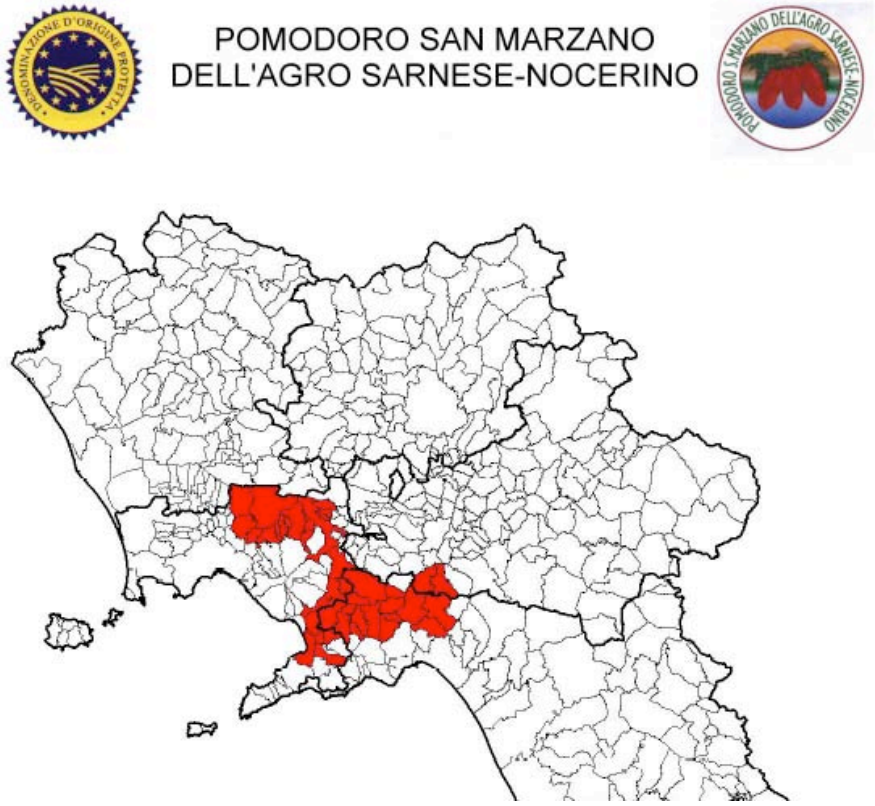

Figure 2

	S. Marzano 619 Sel. ISPORT			Nuovo Super Roma			Nocerino		
	Pear shape index (1-3)	Weight (g)	sblk xly	Pear shape index (1-3)	Weight (g)	sblk xly	Pear shape index (1-3)	Weight (g)	sblk xly
Mean	**1,375**	**64,491**	**0,938**	**2,000**	**53,526**	**0,982**	**1,500**	**33,252**	**0,897**
s_x	0,183	5,472	0,014	0,309	5,546	0,007	0,269	1,698	0,013
C.V.	37,640	24,001	4,363	40,825	27,413	2,002	56,656	16,150	4,643

	S.Marzano L.6			S.Marzano 622 Sel. ISPORT			S.Marzano L.8		
	Pear shape index (1-3)	Weight (g)	sblk xly	Pear shape index (1-3)	Weight (g)	sblk xly	Pear shape index (1-3)	Weight (g)	sblk xly
Mean	**1,714**	**57,611**	**0,820**	**1,500**	**68,645**	**0,879**	**1,625**	**65,971**	**0,840**
s_x	0,286	2,801	0,043	0,342	8,170	0,030	0,183	4,095	0,014
C.V.	44,096	12,863	12,338	55,777	29,152	8,228	31,849	17,558	4,232

	S.Marzano L.4			Malareto Sel. ISPORT			Campano Sel. ISPORT		
	Pear shape index (1-3)	Weight (g)	sblk xly	Pear shape index (1-3)	Weight (g)	sblk xly	Pear shape index (1-3)	Weight (g)	sblk xly
Mean	**1,429**	**61,959**	**0,815**	**1,625**	**66,709**	**0,966**	**1,600**	**56,360**	**0,905**
s_x	0,202	8,591	0,033	0,183	8,207	0,025	0,245	6,655	0,007
C.V.	37,417	36,686	9,131	31,849	34,798	7,436	34,233	26,405	1,788

	S.Marzano 618 Sel. ISPORT			S. Marzano Baldoni			Marzano Sel. ISPORT		
	Pear shape index (1-3)	Weight (g)	sblk xly	Pear shape index (1-3)	Weight (g)	sblk xly	Pear shape index (1-3)	Weight (g)	sblk xly
Mean	**1,500**	**49,794**	**0,887**	**1,625**	**60,570**	**0,860**	**1,750**	**51,145**	**0,908**
s_x	0,267	3,395	0,008	0,183	5,017	0,015	0,250	5,047	0,024
C.V.	50,395	19,284	2,431	31,849	23,427	5,459	40,406	27,911	7,361

	Coimbra			CLX 37203			Red Peel		
	Pear shape index (1-3)	Weight (g)	sblk xly	Pear shape index (1-3)	Weight (g)	sblk xly	Pear shape index (1-3)	Weight (g)	sblk xly
Mean	**1,143**	**65,369**	**0,860**	**1,375**	**67,343**	**0,856**	**1,714**	**102,900**	**0,824**
s_x	0,143	6,976	0,011	0,183	10,143	0,013	0,286	9,516	0,017
C.V.	33,072	28,235	3,299	37,640	42,602	4,459	44,096	24,468	5,312

	PSX 38591			CLX 37201			Alfa 200		
	Pear shape index (1-3)	Weight (g)	sblk xly	Pear shape index (1-3)	Weight (g)	sblk xly	Pear shape index (1-3)	Weight (g)	sblk xly
Mean	**2,000**	**131,680**	**0,853**	**1,714**	**77,600**	**0,888**	**1,429**	**81,649**	**0,849**
s_x	0,000	9,453	0,031	0,184	8,876	0,014	0,202	9,145	0,020
C.V.	0,000	16,052	8,008	28,464	30,262	4,248	37,417	29,633	6,109

	Oskar			Galeon			Italdor		
	Pear shape index (1-3)	Weight (g)	sblk xly	Pear shape index (1-3)	Weight (g)	sblk xly	Pear shape index (1-3)	Weight (g)	sblk xly
Mean	**1,429**	**54,963**	**0,848**	**1,714**	**55,409**	**0,896**	**1,714**	**72,686**	**0,795**
s_x	0,198	8,201	0,018	0,184	6,116	0,012	0,184	9,185	0,016
C.V.	36,417	42,204	6,100	28,464	29,206	3,590	28,464	33,432	5,450

	Corianne			Allflesh 915			Castelong		
	Pear shape index (1-3)	Weight (g)	sblk xly	Pear shape index (1-3)	Weight (g)	sblk xly	Pear shape index (1-3)	Weight (g)	sblk xly
Mean	**1,833**	**88,867**	**0,807**	**2,000**	**65,364**	**0,858**	**1,200**	**70,174**	**0,930**
s_x	0,167	10,389	0,029	0,000	6,852	0,018	0,200	4,757	0,034
C.V.	22,268	28,636	8,733	0,000	31,449	6,239	37,268	15,157	8,161

	Perfect Peel			UC 82			S.Marzano x Tondo		
	Pear shape index (1-3)	Weight (g)	sblk xly	Pear shape index (1-3)	Weight (g)	sblk xly	Pear shape index (1-3)	Weight (g)	sblk xly
Mean	**1,000**	**73,057**	**0,853**	**1,000**	**75,200**	**0,896**	**1,000**	**87,495**	**0,847**
s_x	0,000	3,596	0,020	0,000	12,458	0,013	0,000	4,126	0,012
C.V.	0,000	13,022	6,290	0,000	40,578	3,639	0,000	11,550	3,415

Figure 6

Figure 5

Figure 3

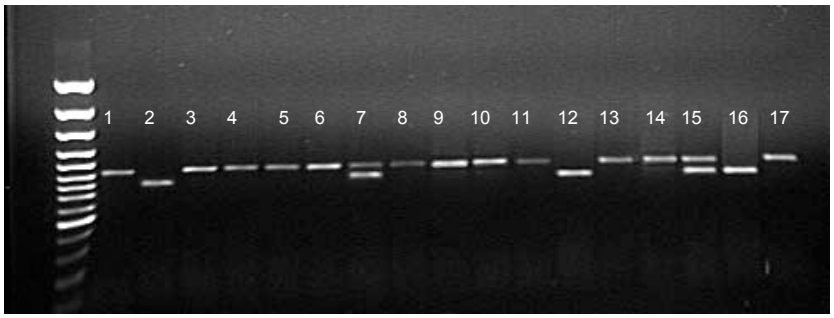

Figure 4