

# DIFFERENTIAL GENETIC STABILITY IN VINEYARDS OF THE CULTIVAR 'ITALY' (*VITIS VINIFERA* L.) CULTIVATED IN DIFFERENT REGIONS OF SOUTHERN AND SOUTHWESTERN BRAZIL

## ESTABILIDADE GENÉTICA DIFERENCIAL EM PARREIRAIS DA CULTIVAR 'ITALY' (*VITIS VINIFERA* L.) CULTIVADA NAS REGIÕES SUL E SUDESTE DO BRASIL

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### SUMMARY

During more than 50 years the vegetative propagation has been the form of maintaining and multiplication of the cv. 'Italy' vineyards, a 'Bicane' x 'Muscat Hamburg' hybrid. In the current study, polymorphism in 17 microsatellite *loci* was used to evaluate the genetic stability at DNA level in vineyards of cv. 'Italy' planted in different regions of the states of Paraná and São Paulo, Brazil. Unchanged and equal allele frequency indicating genetic stability was reported in 47% of the microsatellite *loci* in vineyards of six localities, while allele frequency variation has been observed in *Scu15vv*, *Udv44*, *Udv74*, *Udv96*, *Udv107*, *Udv108*, *Vvmd5*, *Vvmd6* and *Vvs3* microsatellite *loci*. Alleles *Udv96*<sup>140</sup> and *Vvs3*<sup>448</sup>, detected in vines in only one of the vineyards, evidenced somatic mutations at molecular level in cv. 'Italy'. Genetic diversity, as result of changes in the allele frequencies in 53% of microsatellite *loci*, was detected more frequently than somatic mutations due to new alleles. Polymorphism in microsatellite *loci* revealed different genetic stability in vineyards of cv. 'Italy' cultivated in six different Brazilian regions and indicated vineyards with less genetic stability as a possible source of somatic mutants, showing traits of agronomic interest with a potential to generate new cultivars.

### RESUMO

Durante mais de 50 anos a propagação vegetativa tem sido a forma de manter e multiplicar vinhas da cultivar 'Italy', um híbrido entre as variedades 'Bicane' x 'Muscat Hamburg'. No presente estudo, o polimorfismo detectado em 17 *loci* de microssatélites permitiu avaliar a estabilidade genética do DNA em vinhas da cv. 'Italy' plantados em diferentes regiões dos estados do Paraná e São Paulo, Brasil. Frequências alélicas iguais e inalteradas, indicam estabilidade genética, tendo sido reportadas em 47% dos *loci* microssatélites nas vinhas de seis localidades, enquanto variações na frequência de alelos foram observadas nos *loci* *Scu15vv*, *Udv44*, *Udv74*, *Udv96*, *Udv107*, *Udv108*, *Vvmd5*, *Vvmd6* e *Vvs3*. Os alelos *Udv96*<sup>140</sup> e *Vvs3*<sup>448</sup>, detectados em videiras de apenas uma vinha demonstraram mutações somáticas a nível molecular na cv. 'Italy'. A diversidade genética, como resultado de alterações na frequência dos alelos em 53% dos *loci* microssatélites foi mais frequente do que as mutações somáticas, devido ao surgimento de novos alelos. O polimorfismo encontrado nos *loci* microssatélites revelou diferente estabilidade genética nas vinhas da cv. 'Italy' cultivadas nas seis regiões brasileiras e indicou as vinhas com menor estabilidade genética como uma possível fonte de mutantes somáticos, mostrando características de interesse agrônomico com potencial para gerar novas cultivares.

**Key words:** table grapes, clonal polymorphism, genetic divergence, genetic identity.

**Palavras-chave:** uvas de mesa, polimorfismo clonal, divergência genética, identidade genética.

### INTRODUCTION

In the vegetative propagation of grape cultivars, there are several natural mutations at gene level which are linked to skin coloration and which play a significant role in the diversification of grape cultivars (Walker

*et al.*, 2007). A somatic mutation occurring on one side branch of the cv. 'Italy' (VIVC 5582), grown in Santa Mariana, northeastern region of the state of Paraná, Brazil, originated the cv. 'Rubi' (VIVC 22689; Kishino and Mashima, 1980). Another somatic mutation occurring on one side branch of cv.

'Italy' grown in Florai, northwestern region of the state of Paraná, Brazil, gave rise to cultivar 'Benitaka' (VIVC 19816; Sousa, 1996). Further, in 1991, one side branch of cv. 'Benitaka', propagated also in Florai, caused the rise of cv. 'Brasil' (VIVC 19817). In 2006, one side branch of cultivar 'Brasil', propagated in Marialva, northwestern region of the state of Paraná, gave rise to cultivar 'Black Star' (Roberto *et al.*, 2012, 2015, 2017). The cultivar 'Redmeire' also originated from one side branch of cultivar 'Italy' propagated in Marialva, PR Brazil. Differently of 'Italy', the 'Redmeire' fruits are long-ovally shaped, with medium length/width ratio of 1.73, purple coloration and delicate muscatel taste (Pires *et al.*, 2003). Both 'Black Star' and 'Redmeire' cultivars emerged by somatic mutations of 'Brasil' and 'Italy' cultivars, respectively, are not yet cataloged on the *Vitis* International Variety Catalog (VIVC) database. Like this, for more than three decades, several somatic mutations in the genome of the cv. 'Italy' selected several phenotypes which generated at least five new cultivars ('Rubi', 'Benitaka', 'Redmeire', 'Brasil', 'Black Star') of fine table grapes.

The cv. 'Italy' ('Piróvano 65'), which gave rise to three different cultivars ('Rubi', 'Benitaka' and 'Redmeire'), is a hybrid from the cross between 'Bicane' x 'Muscat Hamburg'. It was introduced in the state of São Paulo, Brazil, in 1927 and its culture started in the northeastern region of the state of Paraná (Marialva) in 1962 (Camargo, 1998). For more than fifty years the vegetative propagation has been the form of maintaining and multiplication of cv. 'Italy' vineyards. Consequently, somatic variation has been the primary source of genetic variation employed to produce new phenotypic features. At molecular level, isozymes were used to measure the genetic stability of the introduced cv. 'Italy' (Oliveira-Collet *et al.*, 2005). The lack of allele variation in cv. 'Italy' and in the 'Rubi', 'Benitaka', and 'Brasil' cultivars would indicate apparent genetic stability during the cultivation period. However, high frequency of the null EST-3 carboxylesterase phenotype (61.7%) suggested the occurrence of genetic variations within cv. 'Italy' and their derivatives (Orasmo *et al.*, 2007). High level of polymorphism within and between the cultivars 'Italy', 'Rubi', 'Benitaka' and 'Brasil' (65.1%) were detected by random amplified polymorphic DNA (RAPD) markers, contrasting the hypothesis that the four cultivars were genetically identical (Maia *et al.*, 2009).

Results described by Orasmo *et al.* (2007) and Maia *et al.* (2009) indicate that the genetic diversity of cv. 'Italy' must be high for a cultivar that has been

maintained by vegetative propagation, in contrast to genetic uniformity preliminarily described by Oliveira-Collet *et al.* (2005). The simple sequence repeats (SSR) of DNA in current study were employed to investigate the genetic stability at DNA level in vineyards of cv. 'Italy' maintained in different regions of the states of Paraná and São Paulo, Brazil. SSR markers, also known as microsatellites, proved to be efficient to characterize the genetic diversity between varieties of grapes since the 1990s (Thomas and Scott, 1993; Thomas *et al.*, 1994; Sefc *et al.*, 1999). Although several studies have demonstrated the ineffectiveness of microsatellites to detect genetic diversity within clone collections (Aradhya *et al.*, 2003; Martínez *et al.*, 2006; Pelsy *et al.*, 2010; Emanuelli *et al.*, 2013), microsatellite markers may reveal the level of somatic mutations occurring in vines of cv. 'Italy'.

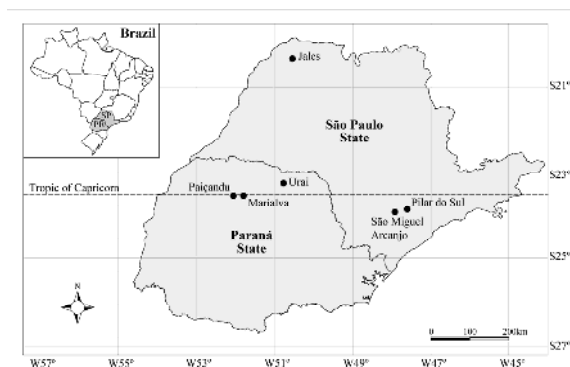
Although mutations that generated the cultivars 'Rubi', 'Benitaka' and 'Redmeire' were described only in the region of Santa Mariana, Florai, and Marialva in the state of Paraná, Brazil, it is possible that not easily identified mutations by morphological characteristics may be occurring in vines of cv. 'Italy' grown in different regions of the country. A high occurrence of somatic mutations may indicate low genetic stability in vineyards of cv. 'Italy', while a low incidence of somatic mutations may indicate greater genetic stability. In addition, information on greater or lesser genetic identity of the vineyards of cv. 'Italy' may be useful for agricultural and cultural practices (nutrition, fertilization, pruning, thinning, among others) of vineyards since plants genetically divergent in a vineyard may respond differently to the same agricultural and cultural practices. Moreover, lesser genetic stability of the vineyards may stimulate greater investment in the search of promising somatic mutations to generate new cultivars. Current hypothesis analyzes whether polymorphism in microsatellite *loci* may reveal a differential genetic stability in vineyards of cv. 'Italy' cultivated in different regions of the states of Paraná and São Paulo, Brazil.

## MATERIAL AND METHODS

### Samples of the cv. 'Italy' of *Vitis vinifera*

DNA was extracted from 69 plants obtained from vineyards in two Brazilian states, namely, Marialva, Paiçandu and Urai in the state of Paraná, and in Jales, Pilar do Sul and São Miguel Arcanjo in the state of São Paulo (Figure 1). Partially expanded leaves without phytosanitary contaminants were collected in 7 to 15 plants in each vineyard. The samples were

individually stored in labelled plastic screen bags to avoid the mixture of vineyards, maintained on ice (4 °C) and transferred to the laboratory. The samples were frozen in liquid nitrogen and stored at -80 °C until DNA extraction.



**Figure 1.** Distribution of the vineyards to form samples representative of cv. 'Italy' (*Vitis vinifera*) that has been maintained by vegetative propagation in two Brazilian states: Paraná (Marialva, Paiçandu and Uraí) and São Paulo (Jales, Pilar do Sul and São Miguel Arcanjo).

*Distribuição dos parreirais para formar as amostras representativas da cultivar 'Italy' (Vitis vinifera) que têm sido mantidas por propagação vegetativa nos dois estados Brasileiros: Paraná (Marialva, Paiçandu e Uraí) e São Paulo (Jales, Pilar do Sul e São Miguel Arcanjo).*

## DNA extraction

DNA was extracted from leaf tissues following Thomas *et al.* (1994) with minor modifications. The modifications included the use of 100 mg of leaves from individual plants replacing the 2.0 g of leaves used in the Thomas and Scott (1993) protocol. After DNA extraction, DNA quantity and quality were determined by 0.8% agar gel electrophoresis buffered with 1x TAE (0.04 M Tris-Acetate and 0.001 M EDTA). A standard DNA ladder ( $\lambda$  phage, 50, 100 and 150 ng) was used as a marker. The gel was stained with 0.5  $\mu\text{g}\cdot\text{mL}$  ethidium bromide, and the image was visualized with a Molecular Image LOCCUS L-PIX - HE (LOCCUS DO BRASIL LTDA., São Paulo SP Brazil) with Picasa 3 software. UV quantification by visual comparison with known quantities of lambda DNA (Invitrogen) averaged between 15 ng/ $\mu\text{L}$  and 200 ng/ $\mu\text{L}$  per sample. After quantification, DNA samples were diluted in a 10 ng/ $\mu\text{L}$  concentration.

## Primer selection and amplification reactions

Thirty-eight SSR primers previously developed for *Vitis vinifera* were used with DNA samples to define the polymorphic primers. After screening the 38 SSR

primers, a set of 17 polymorphic SSR primers, designed Vvs (Thomas and Scott, 1993), VVMD (Bowers *et al.*, 1996, 1999), SCU (Scott *et al.*, 2000), and UDV (Di Gaspero *et al.*, 2005) (Table I), were selected for further analysis. Only 17 primers were polymorphic and used for all 69 plants.

Polymerase chain reaction (PCR) was performed with Techne TC-512 thermal cycler. Amplifications were performed by Touchdown PCR program (Don *et al.*, 1991) using volumes of 20  $\mu\text{L}$  containing 25 ng of genomic DNA, buffer reaction (10 mM Tris-HCl pH 8.8), 2.0 mM of  $\text{MgCl}_2$ , 0.1 mM each of dATP, dGTP, dCTP, dTTP, 0.3  $\mu\text{M}$  of each primer (F and R primers), and 1 unit of Taq DNA polymerase (Invitrogen) and Milli-Q water to bring the reaction to the final volume. Electrophoresis was performed in a 4% MS-8 agar gel using 0.5 x TBE buffer (44.5 mM Tris-borate and 1 mM EDTA) at 60 V, for 4 h. After electrophoresis, the gels were stained with ethidium bromide at 0.5  $\mu\text{g}\cdot\text{mL}$  and images were taken with a Molecular Image LOCCUS L-PIX - HE using Picasa 3 software. The size of PCR fragments was determined with a 100 bp DNA Ladder (Invitrogen).

Polymorphisms from SSR *loci* were analyzed with POPGENE 1.32 (Yeh *et al.*, 1999) to estimate the average number of alleles per *locus*, the frequency of allele in each *locus*, the deficit of heterozygotes, and the genetic divergence between cv. 'Italy' vineyards from the two Brazilian states. Similarity matrix was computed with UPGMA (Sneath and Sokal, 1973), followed by Nei's clustering method (Nei, 1972), with resampling analysis, using 1000 replications. A dendrogram was constructed and drawn from a reference tree by R Development Core Team program (2013) using the adegenet package (Jombart, 2008).

## RESULTS AND DISCUSSION

The analysis of 17 microsatellite *loci* in 69 samples of cv. 'Italy' from six vineyards cultivated in different regions of southern (Paraná state) and southeastern (São Paulo state) Brazil revealed three alleles in *loci* *Udv96* and *Vvs3*, and two alleles in *loci* *Scu10vv*, *Scu11vv*, *Scu15vv*, *Udv26*, *Udv32*, *Udv34*, *Udv40*, *Udv85*, *Udv44*, *Udv74*, *Udv107*, *Udv108*, *Vvmd5*, *Vvmd6*, *Vvmd7* (Table II). Uniform and equal allele frequencies (0.5000) were observed in 47% of the microsatellite *loci* in vines of the six localities (Marialva, Paiçandu, Uraí, Jales, Pilar do Sul and São Miguel Arcanjo). Unchanged and equal allele frequency indicated a genetic stability at the *Scu10vv*, *Scu11vv*, *Udv26*, *Udv32*, *Udv34*, *Udv40*, *Udv85*, and *Vvmd7* *loci* (heterozygous 100%) in vines of the six localities. Since cv. 'Italy' is the result of a cross

between ‘Bicane’ and ‘Moscat Hamburg’ cultivars (Sousa, 1996), the original allele frequency in the hybrid maintained by vegetative propagation has remained unchanged for more than five decades

(since 1962, in Marialva PR Brazil) in 47% of the microsatellite *loci* in vines of the six localities.

**Table I**

Nucleotide sequences of the microsatellite primers, simple sequence repeats of each primer, number of alleles detected by each primer in the cv. ‘Italy’ plants, and variation in allele size (bp) detected in the samples

*Seqüência de nucleotídeos dos primers microssatélites, seqüências simples repetidas de cada primer, número de alelos detetados por cada primer nas plantas da cultivar ‘Italy’, e variação no tamanho dos alelos (pb) detetados nas amostras*

Primer	Nucleotide Sequence (5'→3')	Simple Sequence Repeated	Na	Base Pair
Scu10vv	TACCCCAACACCTTTTCCC <sup>(F)</sup> TTCTCCGCACCTCCTTTTAC <sup>(R)</sup>	(CAA) <sub>6</sub>	2	198-235
Scu11vv	AATTGATAGTGCCACGTTCTCGCC <sup>(F)</sup> AACGCCGACAAGAATCCAAGG <sup>(R)</sup>	(CTT) <sub>8</sub>	2	239-274
Scu15vv	GCCTATGTGCCAGACCAAAAAC <sup>(F)</sup> TTGGAAGTAGCCAGCCCAACCTTC <sup>(R)</sup>	(GAA) <sub>6</sub>	2	174-194
Udv26	GGCTTGTGGATGGGTTTT <sup>(F)</sup> GATGTGTGAGTGCATGTTGC <sup>(R)</sup>	(CT) <sub>n</sub> (CA) <sub>n</sub>	2	138-171
Udv32	CATGCGTATGTGTTAGAGAGCA <sup>(F)</sup> CATGGCATGTGCTTTGTTAT <sup>(R)</sup>	(AC) <sub>n</sub> (AT) <sub>n</sub>	2	139-183
Udv34	AAGAGACCAAGGATAGATCAAACA <sup>(F)</sup> AAATGCAACGGGAGATGGTA <sup>(R)</sup>	(TG) <sub>n</sub> (AG) <sub>n</sub> (AT) <sub>n</sub> (AGAGG) <sub>n</sub>	2	168-190
Udv40	GTATGGGTGGGTGGGTGTGT <sup>(F)</sup> GGTAACTGGTGTGGGAATCAA <sup>(R)</sup>	(TG) <sub>n</sub> (AG) <sub>n</sub>	2	143-171
Udv44	GGAACTATCAACCCGACCAA <sup>(F)</sup> CATTCCAACACATGGGTCA <sup>(R)</sup>	(TG) <sub>n</sub>	2	113-146
Udv74	GCCAGGGAAGTTGGTTCTCT <sup>(F)</sup> TGCAATGTGTTTTGCACAAG <sup>(R)</sup>	(CT) <sub>n</sub> (CA) <sub>n</sub>	2	299-327
Udv85	CTCCCCAGTAACCATGGAAG <sup>(F)</sup> CACTGGATGAGATGGCAGAA <sup>(R)</sup>	(AC) <sub>n</sub> (AT) <sub>n</sub> (AG) <sub>n</sub>	2	128-143
Udv96	CTTCCCCAAGCTCTATT <sup>(F)</sup> CGGAGAGTTGCGAGTGACTA <sup>(R)</sup>	(CA) <sub>n</sub> (TA) <sub>n</sub>	3	107-140
Udv107	GGAAAAGGACACAAAAGTAGCAA <sup>(F)</sup> CAGAATACCCAGTTGGTT <sup>(R)</sup>	(CT) <sub>n</sub> (CA) <sub>n</sub> (TA) <sub>n</sub>	2	146-171
Udv108	TGTAGGGTCCAAAGTTCAGG <sup>(F)</sup> CCTTTTTATATGTGGTGGAGCA <sup>(R)</sup>	(CT) <sub>n</sub> (CA) <sub>n</sub> (GT) <sub>n</sub>	2	193-209
Vvmd5	CTAGAGCTACGCCAATCCAA <sup>(F)</sup> TATACAAAAATCATATTCCTAAA <sup>(R)</sup>	(CT) <sub>3</sub> AT(CT) <sub>11</sub> ATAG(AT) <sub>3</sub>	2	239-279
Vvmd6	ATCTTAACCTAAAACCAT <sup>(F)</sup> CTGTGCTAAGACGAAGAAGA <sup>(R)</sup>	(CT) <sub>4</sub> C(CT) <sub>2</sub> TTAG(CT)TAAT(CT) <sub>6</sub> C(CT) <sub>2</sub> C(CT) <sub>2</sub>	2	180-199
Vvmd7	AGAGTTGCGGAGAACAGGAT <sup>(F)</sup> CGAACCTTACACGCTTGAT <sup>(R)</sup>	(CT) <sub>14.5</sub>	2	253-298
Vvs3	TGCCCTATCAATTAGTTCACCTA <sup>(F)</sup> TCGACTTTGATATATTGATGATT <sup>(R)</sup>	(GT) <sub>6</sub>	3	190-448

In contrast, allele frequency variation has been observed in *Scu15vv*, *Udv44*, *Udv74*, *Udv96*, *Udv107*, *Udv108*, *Vvmd5*, *Vvmd6*, and *Vvs3* microsatellite *loci*. For instance, the frequency of *Udv96*<sup>107</sup> and *Udv96*<sup>115</sup> alleles is different from 0.5000 and different in vines cultivated in the six regions of southern and southeastern Brazil (Table II). In addition, a third allele was detected at *Udv96* (*Udv96*<sup>140</sup>) and *Vvs3* (*Vvs3*<sup>448</sup>) *loci* in a cv. ‘Italy’ vine from Paiçandu. Different allele frequencies in *Scu15vv*, *Udv34*, *Udv107*, *Udv108*, *Vvmd5* and *Vvmd6* *loci* have

conferred different level of mean heterozygosity for the vines of the six regions (Table III). Mean observed heterozygosity (Ho) was higher than mean expected heterozygosity (He) rates and ranged from Ho = 0.7000 in vines of Paiçandu to Ho = 0.9647 in the vines of São Miguel Arcanjo. Highest He rates were estimated in the vines from Jales (He = 0.4950) and São Miguel Arcanjo (He = 0.4949), whereas the lowest rates were detected in vines from Urai (He = 0.4064) and Paiçandu (He = 0.4085) (Table III).

Table II

Allele frequencies in microsatellite loci of the 'Italy' cultivar (*Vitis vinifera*) from six localities: Jales (J1-J15), São Miguel Arcanjo (SM16-SM30), Pilar do Sul (PS31-PS37), Uraí (U38-U52), Marialva (M53-M59), and Paçandú (P60-P69)

Frequência de alelos nos locos microssatélites da cultivar 'Italy' das seis localidades: Jales (J1-J15), São Miguel Arcanjo (SM16-SM30), Pilar do Sul (PS31-PS37), Uraí (U38-U52), Marialva (M53-M59), e Paçandú (P60-P69)

Loci	Scu10 vv	Scu 11vv	Scu15 vv	Udv2 6	Udv3 2	Udv3 4	Udv 40	Udv4 4	Udv 74	Udv 85	Udv9 6	Udv1 07	Udv1 08	Vvm d5	Vvm d6	Vvm d7	Vvs3
J1	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/327	128/143	107/115	146/146	193/209	239/279	180/199	253/298	190/218
J2	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/327	128/143	107/115	146/171	193/209	239/279	180/199	253/298	190/218
J3	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/327	128/143	107/115	146/146	193/209	239/279	180/199	253/298	190/218
J4	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/327	128/143	107/115	146/171	193/209	239/279	180/199	253/298	190/218
J5	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/327	128/143	107/115	146/171	193/209	239/279	180/199	253/298	190/218
J6	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/327	128/143	107/115	146/171	193/209	239/279	180/199	253/298	190/218
J7	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/327	128/143	107/115	146/171	193/209	239/279	180/199	253/298	190/218
J8	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/327	128/143	107/115	146/171	193/209	239/279	180/199	253/298	190/218
J9	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/327	128/143	107/115	146/146	193/209	239/279	180/199	253/298	190/218
J10	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/327	128/143	107/115	146/171	193/209	239/279	180/199	253/298	190/218
J11	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/327	128/143	107/115	146/146	193/209	239/279	180/199	253/298	190/218
J12	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/327	128/143	107/115	146/171	193/209	239/279	180/199	253/298	190/190
J13	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/327	128/143	107/115	146/171	193/209	239/279	180/199	253/298	190/218
J14	198/235	239/274	174/174	138/171	139/183	168/190	143/171	113/146	299/327	128/143	107/107	146/171	193/209	239/239	180/180	253/298	190/218
J15	198/235	239/274	174/174	138/171	139/183	168/190	143/171	113/146	299/299	128/143	107/115	146/146	193/209	239/279	180/180	253/298	190/218
SM16	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/327	128/143	115/115	146/171	193/209	239/279	180/180	253/298	190/218
SM17	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/327	128/143	107/115	146/171	193/209	239/279	180/199	253/298	190/218
SM18	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/327	128/143	107/107	146/171	193/193	239/279	180/199	253/298	190/218
SM19	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/327	128/143	107/115	146/171	193/209	239/279	180/199	253/298	190/218
SM20	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/327	128/143	107/115	146/171	193/209	239/279	180/199	253/298	190/218
SM21	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/327	128/143	107/115	146/171	193/209	239/279	180/199	253/298	190/218
SM22	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/327	128/143	107/115	146/171	193/209	239/279	180/199	253/298	190/218
SM23	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/327	128/143	115/115	146/171	193/209	239/279	180/199	253/298	190/218
SM24	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/327	128/143	115/115	146/171	193/209	239/279	180/199	253/298	190/218
SM25	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/327	128/143	115/115	146/171	193/209	239/279	180/199	253/298	190/218
SM26	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/327	128/143	115/115	146/171	193/209	239/279	180/199	253/298	190/218
SM27	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/327	128/143	115/115	146/146	193/209	239/279	180/199	253/298	190/218
SM28	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/327	128/143	115/115	146/171	193/209	239/279	180/199	253/298	190/218
SM29	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/327	128/143	115/115	146/171	193/209	239/279	180/199	253/298	190/218
SM30	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/327	128/143	115/115	146/171	193/209	239/279	180/199	253/298	190/218
PS31	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/327	128/143	107/115	146/146	193/209	239/279	180/199	253/298	190/218
PS32	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/327	128/143	107/115	146/171	193/209	239/279	180/199	253/298	190/218
PS33	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/327	128/143	107/115	146/171	193/209	239/279	180/199	253/298	190/218
PS34	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/327	128/143	107/107	146/146	193/209	239/279	180/199	253/298	190/218
PS35	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/327	128/143	107/115	146/146	193/209	239/279	180/199	253/298	190/218
PS36	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/113	299/327	128/143	107/107	146/146	193/193	239/279	180/180	253/298	190/218
PS37	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/113	299/327	128/143	107/107	146/171	193/193	239/279	180/180	253/298	190/218
U38	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/327	128/143	107/115	146/171	193/209	239/279	180/180	253/298	190/218
U39	198/235	239/274	174/174	138/171	139/183	168/190	143/171	113/146	299/327	128/143	107/115	146/146	193/193	239/239	180/180	253/298	190/190
U40	198/235	239/274	174/174	138/171	139/183	168/190	143/171	113/146	299/327	128/143	107/115	146/146	193/193	239/279	180/199	253/298	190/218
U41	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/327	128/143	107/115	146/171	193/209	239/279	180/199	253/298	190/190
U42	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/327	128/143	107/115	146/171	193/193	239/279	180/180	253/298	190/190
U43	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/327	128/143	107/115	146/146	193/193	239/279	180/199	253/298	190/218
U44	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/327	128/143	107/115	146/171	193/209	239/279	180/180	253/298	190/190
U45	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/327	128/143	107/115	146/146	193/193	239/279	180/180	253/298	190/190
U46	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/327	128/143	115/115	146/171	193/209	239/279	180/199	253/298	190/218
U47	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/327	128/143	115/115	146/171	193/209	239/279	180/180	253/298	190/218
U48	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/299	128/143	115/115	146/171	193/209	239/279	180/199	253/298	190/190
U49	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/327	128/143	115/115	146/171	193/193	239/279	180/180	253/298	190/190
U50	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/299	128/143	107/115	146/146	193/193	239/279	180/180	253/298	190/190
U51	198/235	239/274	174/174	138/171	139/183	168/190	143/171	113/146	299/327	128/143	115/115	146/171	193/193	239/279	180/180	253/298	190/190
U52	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/299	128/143	115/115	146/171	193/193	239/279	180/199	253/298	190/218


  
**1-15 J - Jales**
  
**16-30 SM - São Miguel Arcanjo**
  
**31-37 PS - Pilar do Sul**
  
**38-52 U - Uraí**
  
**53-59 M - Marialva**
  
**60-69 P - Paçandú**

**Table II**  
(Continuation)  
(Continuação)

Loci	Scu1 0vv	Scu 11vv	Scu15 vv	Udv 26	Udv 32	Udv 34	Udv 40	Udv 44	Udv 74	Udv 85	Udv 96	Udv 107	Udv 108	Vvm d5	Vvm d6	Vvm d7	Vvs3
M53	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/327	128/143	115/115	146/171	193/209	239/279	180/199	253/298	190/218
M54	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/327	128/143	115/115	146/171	193/209	239/279	180/199	253/298	190/218
M55	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/327	128/143	115/115	146/171	193/209	239/279	180/180	253/298	190/218
M56	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/327	128/143	107/115	146/171	193/209	239/279	180/199	253/298	190/218
M57	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/299	128/143	107/115	146/171	193/209	239/279	180/199	253/298	190/218
M58	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/299	128/143	107/107	146/171	193/209	239/279	180/180	253/298	190/218
M59	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/146	299/299	128/143	107/115	146/171	193/209	239/279	180/180	253/298	190/218
P60	198/235	239/274	174/174	138/171	139/183	168/190	143/171	113/113	299/327	128/143	107/115	146/171	193/209	239/279	180/180	253/298	190/448
P61	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/113	299/327	128/143	115/115	146/146	193/193	239/279	180/180	253/298	448/448
P62	198/235	239/274	174/174	138/171	139/183	168/190	143/171	113/113	299/327	128/143	140/140	146/146	193/209	239/239	180/180	253/298	448/448
P63	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/113	299/327	128/143	115/115	146/171	193/209	239/279	180/180	253/298	190/448
P64	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/113	299/327	128/143	115/115	146/171	209/209	239/279	180/180	253/298	448/448
P65	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/113	299/327	128/143	115/115	146/171	193/209	239/279	180/180	253/298	448/448
P66	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/113	299/327	128/143	115/115	146/171	193/209	239/279	180/180	253/298	190/448
P67	198/235	239/274	174/174	138/171	139/183	168/190	143/171	113/113	299/327	128/143	115/115	146/171	209/209	239/239	180/180	253/298	448/448
P68	198/235	239/274	174/174	138/171	139/183	168/190	143/171	113/113	299/327	128/143	115/115	146/171	209/209	239/279	180/180	253/298	448/448
P69	198/235	239/274	174/194	138/171	139/183	168/190	143/171	113/113	299/327	128/143	115/115	146/171	193/209	239/279	180/180	253/298	190/448



**Table III**

Number of alleles (Na) and number of effective alleles (Ne) per polymorphic locus, mean observed heterozygosity ( $H_o$ ) and expected heterozygosity ( $H_e$ ) in microsatellite loci of cv. 'Italy' of *Vitis vinifera* from six vineyards in two Brazilian states: Paraná (PR) and São Paulo (SP)

*Número de alelos (Na) e número efetivo de alelos (Ne) por loco polimórfico, heterozigosidade média observada ( $H_o$ ) e heterozigosidade média esperada ( $H_e$ ) nos locos microsatélites da cultivar 'Italy' de *Vitis vinifera* dos seis parreirais em dois estados Brasileiros: Paraná (PR) e São Paulo (SP)*

Sample	Na	Ne	$H_o$	$H_e$
Marialva, PR	2.0	1.9546	0.9160	0.4868
Paçandu, PR	1.94	1.7838	0.7000	0.4085
Uraí, PR	2.0	1.7548	0.7020	0.4064
Pilar do Sul, SP	2.0	1.9261	0.8908	0.4778
São Miguel Arcanjo, SP	2.0	1.9822	0.9647	0.4949
Jales, SP	2.0	1.9815	0.9451	0.4950
Mean	2.11	1.9763	0.8525	0.4923

Different allele frequencies in nine microsatellites of cv. 'Italy' estimated a moderate genetic divergence ( $0.05 < F_{ST} < 0.15$ ) between the vines of Marialva, Paçandu, Uraí, Jales, Pilar do Sul and São Miguel Arcanjo. Genetic divergence among the six vineyards resulted in  $F_{ST} = 0.0648$ .

In spite of the somatic mutations leading to new alleles ( $Udv96^{140}$  and  $Vvs3^{448}$ ) and in spite of changes in the allele frequencies in 53% of microsatellite loci,

the similarity coefficient (Nei, 1978) between the vineyards ranged between 0.8520 (between Uraí and Paçandu) and 1.000 (between Jales and Pilar do Sul, Jales and São Miguel Arcanjo, Jales and Marialva, Pilar do Sul and Marialva, and São Miguel Arcanjo and Marialva) (Table IV). High genetic identity indicates a common origin for the vineyards of cv. 'Italy' introduced in the state of São Paulo and its culture in the north of the state of Paraná (Camargo, 1994). Genetic identity rates are important for winegrowers to foreground cultural practices in vineyards. In the case of vineyards with 100% identity rates, such as the samples analyzed in current study, it may be possible, for instance, to adopt similar options and procedures for the control of pathogens infection.

The dendrogram generated by Nei's coefficient from the analysis of individual vines of cv. 'Italy' (constructed from 17 microsatellite primers data using adegenet package from R Development Core Team, 2013) identified two well-defined larger groups and two smaller groups formed by vines from six cv. 'Italy' vineyards (Figure 2). Dendrogram showed several homogeneous groups formed by vines from São Miguel Arcanjo (80%), Jales (60%), Uraí (53%), and Paçandu (80%) vineyards. Heterogeneous groups were also formed by mixture of vines from different vineyards. Genetic identity in the dendrogram was evident between 60% of the vines of the same or of different vineyards. Vines from Jales, Pilar do Sul, Uraí, Marialva, and São Miguel Arcanjo vineyards

showed genetic identity, so that the six ‘Italy’ vineyards represented a moderate genetic divergence.

**Table IV**

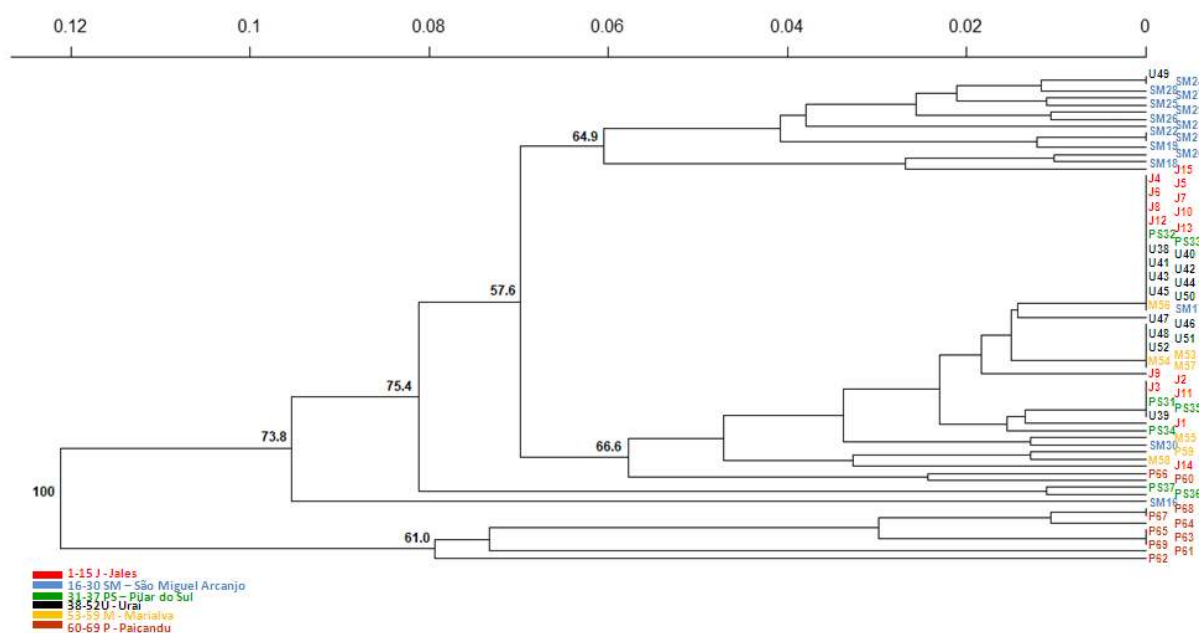
Nei's genetic identity (1978) between vineyards of ‘Italy’ (*Vitis vinifera* L.) cultivated in different regions of Southern (Marialva, Paçandu, Urai) and Southwest Brazil (Pilar do Sul, São Miguel Arcanjo, Jales) as revealed by 17 microsatellite loci

*Identidade genética de Nei (Nei, 1978) entre os parreirais de uvas ‘Italy’ (Vitis vinifera L.) cultivadas nas regiões Sul (Marialva, Paçandu, Urai) e Sudeste (Pilar do Sul, São Miguel Arcanjo, Jales) do Brasil, revelada pelos 17 locos microssatélites*

	Marial- va	Paçan- du	Urai	Pilar do Sul	São Miguel Arcanjo	Jales
<b>Marialva</b>	---	0.9189	0.9625	1.0000	1.0000	1.0000
<b>Paçandu</b>		---	0.8520	0.8902	0.9026	0.8904
<b>Urai</b>			---	0.9345	0.9451	0.9474
<b>Pilar do Sul</b>				---	0.9932	1.0000
<b>São Miguel Arcanjo</b>					---	1.0000
<b>Jales</b>						---

Occurrence of new alleles with definite frequencies was detected only at *Udv96* and *Vvs3* loci in the cv.

‘Italy’ from Paçandu (Table II). However, an extra allele observed as having an intensity comparable with the two original alleles was evident in two plants of cv. ‘Italy’ from Urai and four plants from São Miguel do Iguçu using the UDV107 primer. The primer SCU10VVW also detected a third allele in three plants of cv. ‘Italy’ from São Miguel Arcanjo, six plants from Marialva, and two plants from Paçandu. The extra alleles observed as having an intensity comparable with the two original alleles defined a tri-allelic pattern at *Udv107* and *Scu10vvw* microsatellite loci. The tri-allelic phenotype was not considered at analysis of polymorphism in the SSR loci of cv. ‘Italy’, but is important to highlight a third allele extra at SSR locus since the detection of three alleles at the same locus could be the result of a chimeric structure in which genotypes of different layers of cells displayed different alleles. The tri-allelic pattern in SSR loci have been considered an evidence of chimeras, a mechanism of genetic diversification well characterized by Hocquigny *et al.* (2004) occurring in grapevines clones (Crespan, 2004; Moncada *et al.*, 2006). In cv. ‘Italy’ vines the chimeras were evident in 13%, 46%, 85%, and 20% of the plants from Urai, São Miguel Arcanjo, Marialva, and Paçandu, respectively.



**Figure 2.** Dendrogram generated by Jaccard coefficient from analysis of individual vines of the six cv. ‘Italy’ vineyards from: Urai (U), São Miguel Arcanjo (SM), Jales (J), Pilar do Sul (PS), Marialva (M), and Paçandu (P) in Paraná (U, M, P) and São Paulo (SM, J, PS) States of Brazil based on 17 microsatellite markers. Numbers beside nodes indicate relative bootstrap frequencies (%).

*Dendrograma gerado pelo coeficiente de Jaccard a partir da análise de plantas individuais dos seis parreirais da cultivar ‘Italy’: Urai (U), São Miguel Arcanjo (SM), Jales (J), Pilar do Sul (PS), Marialva (M), e Paçandu (P) nos estados do Paraná (U, M, P) e São Paulo (SM, J, PS), Brasil, baseado nos 17 marcadores microssatélites. Os números junto aos nós indicam a frequência relativa (%) do bootstrap.*

High level of mean observed heterozygosity in microsatellite *loci* is expected for cv. 'Italy' as a product of the cross between the 'Bicane' and 'Moscat Hamburg' cultivars (Sousa, 1996). Most grape cultivars are highly heterozygous (Di Vecchi-Staraz *et al.*, 2009; This *et al.*, 2011) due to vegetative (clonal) propagation to maintain the selected genotypes. High  $H_o$  rates have been reported in cultivars of Central Europe ( $H_o = 0.776 - 0.904$ ; Sefc *et al.*, 2000) and Spain ( $H_o = 0.756 - 0.909$ ; Martín *et al.*, 2003, and  $H_o = 0.744 - 0.949$ ; Fernández-González *et al.*, 2007), in cultivars of Tunisia ( $H_o = 0.71 - 0.98$ ; Snoussi *et al.*, 2004) and also in different species of *Vitis* ( $H_o = 0.68 - 0.92$ ; Fernández *et al.*, 2008). High rates of observed heterozygosity ( $H_o = 0.743 - 0.857$ ; Carimi *et al.*, 2010, and  $H_o = 0.78 - 0.92$ ; Alba *et al.*, 2014, respectively) were reported in genotypes of Italian grapevines and Italian table grapes (cultivars and candidate cultivars). Lower rates with greater interval were reported in cultivars in Portugal ( $H_o = 0.60 - 0.97$  and  $0.55 - 0.91$ ; Lopes *et al.*, 1999, 2006), in North America ( $H_o = 0.30 - 1.0$ ; Pollefeys and Bousquet, 2003), in hybrids of France ( $H_o = 0.0 - 1.0$ ; Merdinoglu *et al.*, 2005), in Peruvian and Argentine Criollas varieties ( $H_o = 0.615 - 1.000$ ; Martínez *et al.*, 2006), and in Iranian grape cultivars ( $H_o = 0.49 - 0.97$ ; Doulati-Baneha *et al.*, 2013). High rates of observed heterozygosity in the cv. 'Italy' ( $H_o = 0.7000 - 0.9647$ ; Table III) agrees to the highest  $H_o$  values reported for cultivars of Central Europe, Spain, Tunisia, and different Italian genotypes and species of *Vitis*.

Somatic mutations are also expected in the vegetative propagation of cv. 'Italy'. Alleles *Udv96*<sup>140</sup> and *Vvs3*<sup>448</sup> detected only in the vines of Paiçandu (Table II) evidence somatic mutations at molecular level in cv. 'Italy'. Somatic mutations in microsatellite *loci* of clones are more often detected by screening large numbers of *loci* and clones. Riaz *et al.* (2002) detected intra-variety polymorphism in 17 microsatellite markers when 100 markers were genotyped in more than 20 clones each for 'Pinot noir' and 'Chardonnay'. Higher polymorphism was detected in 49 microsatellites of 145 clones of 'Pinot' cultivar (22.4%; Hocquigny *et al.*, 2004) and in 84 microsatellite *loci* of 59 clones of 'Cabernet Sauvignon' (21.4%; Moncada *et al.*, 2006). Current authors detected somatic mutations in two microsatellite *loci* in cv. 'Italy' after screening of only 17 *loci* in six clones: a polymorphism of 11.8%, which was consistent with rate described by Moncada and Hinrichsen (2007) in the analysis of 20 *loci* in 25 clones of 'Carmenère' (10%) from Chile, France and Italy.

On the other hand, the polymorphism at microsatellite *loci* of the cv. 'Italy' may be higher than 11.8% if the chimeras pattern is considered since the tri-allelic pattern at microsatellite *loci* have been proposed as a mechanism of genetic diversification in grapevines clones (Crespan, 2004; Hocquigny *et al.*, 2004; Moncada *et al.*, 2006), which have vegetative propagation. The tri-allelic pattern at *Udv107* and *Scu10vv* *loci* has been observed in 24% of vines in cv. 'Italy' from Uraí (13%), São Miguel Arcanjo (46%), Marialva (85%), and Paiçandu (20%). The presence of a third allele in SSR *locus* is an indicative of these *loci* potential in molecular diversification and variability. Thus, in addition to the somatic mutation in *Udv96* and *Vvs3* *loci* the detection of extra alleles may be conferring high genetic diversity also for the *Udv107* and *Scu10vv* *loci* in cv. 'Italy' vines cultivated in the six regions of southern and southeastern Brazil. The cause and effects of somatic mutations at microsatellite *loci* of cv. 'Italy' are unknown and there are doubts if mutations are related to the age of the vineyards. Somatic mutation in *Udv96* and *Vvs3* *loci* does not seem to be more frequent in the oldest vineyards. It was not possible to know the age of the vineyards established in Uraí, Jales, Pilar do Sul and São Miguel Arcanjo. However, in Paiçandu where somatic mutations occurred at *Udv96* and *Vvs3* *loci*, the cv. 'Italy' vineyards were established in 1997, or rather, 35 years after the start of vineyard cultivation in Marialva in 1962 (M. Collet, personal information). The oldest vineyards of cv. 'Italy' in Marialva showed greater genetic stability than the most recently vineyards established in Paiçandu. In contrast, the chimeras were more evident in vines from Marialva (85%). It may be supposed that the older varieties were exposed for a longer time to environmental stress and therefore they had a great chance to accumulate more mutations (Pelsy, 2010).

The genetic potential of Paiçandu vineyards should be underscored. Somatic variants potentially represent a commercial interest if they improve an important agronomical trait (e.g., height, yield, pathogen susceptibility and others) or provide innovation of the product (This *et al.*, 2011). The vineyards of Paiçandu with low genetic stability (greater genetic variability) should be more carefully investigated as a source of somatic mutants with favorable agronomic traits. Somatic mutations contribute towards the generation of genetic variation which may be relevant for improvements. They may eventually give rise to plant variants without going through the gametophytic phase. Somatic variation has a strong relevance in the generation of the cultivars 'Rubi', 'Benitaka',



‘Brasil’, ‘Redmeire’ and ‘Black Star’ (Kishino and Mashima, 1980; Sousa, 1996; Pires *et al.*, 2003; Roberto *et al.*, 2012) from cv. ‘Italy’ as the fundamental genome.

Genetic diversity as a result of changes in allele frequencies in 53% of the microsatellite *loci* (*Scu15vv*, *Udv44*, *Udv74*, *Udv96*, *Udv107*, *Udv108*, *Vvmd5*, *Vvmd6*, and *Vvs3 loci*) was more frequently detected than somatic mutations due to new alleles. Changes in allele frequencies occurred at different *loci* in different cv. ‘Italy’ vineyards of the six regions (Table II). Several factors may be related with evidences of changes in allele frequencies in microsatellite *locus*, including the random selection of genotypes (homozygous or heterozygous for microsatellite *loci* here analyzed) for the establishment of a new vineyard by vegetative propagation. Different values of  $H_o$  for the same variety multiplied by vegetative propagation on the two or more cultivated areas may be due to different genotypes of cv. ‘Italy’ used in the foundation of the cultivated areas. Other plausible hypothesis to explain different values of  $H_o$  for the same variety multiplied by vegetative propagation may be that plants identified as homozygous in the microsatellite *loci* in vineyards of the six regions may contain null alleles masking the identification of the original phenotype reported as heterozygote. Somatic mutations may lead to the appearance of null alleles and mutations in sequences of microsatellite flanking regions of grapes were confirmed by sequencing (Fernández *et al.*, 2008) and associated to the emergence of null alleles. High frequency of null EST-3 carboxylesterase phenotype (52.3%) has been suggested in cvs. ‘Italy’ (Orasmo *et al.*, 2007). Null alleles in microsatellite *loci* have also been reported in varieties and cultivars of grapes from Portugal (Lopes *et al.*, 1999), Spain (Martín *et al.*, 2003) and in North American hybrids and in cultivars from France (Pollefeys and Bousquet, 2003).

The occurrence of somatic crossing-over events is another factor that may lead to changes in the frequency of alleles in microsatellite *locus* with the emergence of homozygous phenotypes. The somatic recombination induced by retrotransposons was used to explain changes in frequency for berry skin color in the colored (‘Benitaka’ and ‘Brasil’) cv. ‘Italy’ (Oliveira-Collet *et al.*, 2005) and in the high frequency of the null EST-3 carboxylesterase phenotype within cv. ‘Italy’ (Orasmo *et al.*, 2007). For the ‘Benitaka’ cultivar, Azuma *et al.* (2009) showed that color recovery was due to a new allelic form of *VvmybA1* gene formed by illegitimate homologous recombination between *VvmybA1* and *VvmybA3* genes that originated the *VvmybA1*<sup>BEN</sup>

allele. Somatic crossing-over events in microsatellite *loci* may have caused the apparently different random frequencies of alleles in the nine microsatellite *loci* (*Scu15vv*, *Udv44*, *Udv74*, *Udv96*, *Udv107*, *Udv108*, *Vvmd5*, *Vvmd6*, and *Vvs3 loci*) of cv. ‘Italy’ from Marialva, Paçandu, Uraí, Jales, Pilar do Sul and São Miguel Arcanjo (Table II).

Double-strand breaks in DNA leading to the occurrence of somatic recombination may be caused by external factors in response of pathogen attack and/or poisons used to their control, or by internal factors during replication and transposon excision and even in response to intrinsic metabolic activities induced by external factors (Schuermann *et al.*, 2005). Geminivirus infection in experimental conditions also promoted somatic homologous recombination (Richter *et al.*, 2014). Consequently, the exposition of leaves of cv. ‘Italy’ in different types and concentrations of fungicides and poisons (frequently applied for disease control; M. Collet, personal information) may induce point mutations and/or chromosomal breaks that contribute towards the enhancement of different frequencies of alleles in microsatellite *loci* of cvs ‘Italy’ in different regions. The reaction of fungicides in inhibiting seven carboxylesterases from different *loci* in the leaves of cv. ‘Italy’ was described by Orasmo *et al.* (2015).

The emergence of the new alleles *Udv96*<sup>140</sup> and *Vvs3*<sup>448</sup> beside of changes in allele frequencies detected at the *loci* *Scu15vv*, *Udv44*, *Udv74*, *Udv96*, *Udv107*, *Udv108*, *Vvmd5*, *Vvmd6*, and *Vvs3* have leading to a moderate genetic divergence among the six vineyards of cv. ‘Italy’ maintained by vegetative propagation. Low rates between cultivated grapevine populations have been commonly related with  $F_{ST}$  pairwise rates ranging between 0.0224 (Eastern-Western Europe) and 0.0374 (Western Europe-North Africa). Results showed higher  $F_{ST}$  pairwise rates only when the wild population was compared to other populations of cultivated grapevines. The highest genetic differentiation rate (0.0747) was recorded between wild accessions and North African cultivars (Riahi *et al.*, 2012). A moderate genetic divergence among the vineyards of Italy coupled to the somatic variation may be a very powerful tool to improve the classical cv. ‘Italy’ and a good insight for producers of new and different varieties. The preservation of the inter- and intra-vineyards diversity must be a goal for producers interested in the selection and use of somatic variation.

Although several studies have demonstrated the ineffectiveness of some microsatellites when genetic diversity within clone collections is addressed (Aradhya *et al.*, 2003; Martínez *et al.*, 2006; Pelsy *et al.*

*al.*, 2010; Emanuelli *et al.*, 2013), current study proved the effectiveness of microsatellites for clonal polymorphism in vegetative propagation of the cv. Italy, due to the somatic variation leading to new alleles and changes in allele frequencies. Polymorphism in microsatellite *loci* revealed different genetic stability in vineyards of cv. 'Italy' cultivated in six different regions of Brazil. It actually indicates vineyards with low genetic stability as a possible source of somatic mutants revealing traits of agronomic interest capable of generating new cultivars. Furthermore, the microsatellite primers selected in the present study could be used to investigate the genetic stability of somatic mutants derived from the cultivar 'Italy': 'Rubi', 'Benitaka', 'Brasil', 'Black Star' and 'Redmeire'.

## CONCLUSIONS

The microsatellite markers were effective to evidence somatic mutations and alterations in allele frequencies in the SSR *loci* that led to a moderate genetic divergence among the vineyards of the cultivar 'Italy'

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maintained by vegetative propagation in the south and southeast regions of Brasil. Polymorphism in microsatellite *loci* was also useful to identify the genetically more stable vineyards (from Jales and Pilar do Sul) and to estimate the genetic identity among the vineyards of the cv. 'Italy' cultivated in the six localities of the south and southeast regions of Brasil. A prospect for evolution due to somatic mutations in 'Italy' cultivar was more evident in the vineyard of Paiçandu due to the appearance of two new alleles with defined frequencies. Also, evidence of chimeras in the vineyards of Urai, São Miguel Arcanjo, Marialva, and Paiçandu may be highlighted as potential mechanisms of genetic diversification acting as a probable evolutionary force in 66.6% of the investigated vines.

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