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Phenotypic divergence of grapes using productive cycle

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Abstract

Classifying vines regarding the number of days required to complete de productive cycle and the duration of intermediate phenologic phases are essential for genetic improvement programs, implementation of cultivation techniques and handling of the grape harvest. The objective of this study was to evaluate the access phenotypic divergence of grapes from the germplasm collection by the Agronomic Institute - IAC. Evaluations were performed for 4 years of production, from 2012 to 2015, when the plants were 4 years old. The training system applied the espalier, at a 2.0 x 1.0 m spacing between lines and plants, respectively. The IAC's grape germplasm collection comprised 110 varieties of the *Vitis vinífera*, *Vitis labrusca* species and inter-specific hybrids grafted into the IAC 766 rootstocks, being 3 plants per each variety. After the pruning of the main vine phonologic phases, the number of days was evaluated using the scale proposed by Eichhorn and Lorenz. Two evaluations a week were carried out until the flowering, and afterwards one evaluation was performed a week, the period between pruning and the beginning of sprouting, full flowering with 50% flowers opened, beginning of maturation (*veraison*) and maturation (harvest), visually mature fruits and content of soluble solids above 14ºBrix. Multivariate analysis, such as the correlation between varieties, analysis of the main components (PCA) and methods of non-weighted arithmetic means (UPGMA) was applied to classify the phenotypes according to the productive cycle. A relation was found between the sprouting and the flowering phases, as well as the starting maturation and maturation. The first two components explained 81% of the total variability, being that the starting maturation and maturation were the best variables to study the divergence of vine phenotypes. Also, combining the UPGMA method and the PCA analysis that distinguished three groups, allowed us to divide the phenotypes into 25 processes, 75 median and 10 late varieties, according to their productive cycle. It was possible to conclude that the techniques used to study the genetic diversity applied to phenologic characters were effective to evaluate the vines phenotypic divergence, and therefore, the multivariate analysis may be used to guide future vine improvement programs.

Keywords: phenology, genetic resources, germplasm, plant breeding, variability.

Introduction

Grape is a very important culture in the world because of the production of wine, juice, fresh and dried fruits (Migicovsky et al., 2016). But similar to all perennial species, it needs a considerably long time to develop new varieties since maturity often requires several growing seasons until breeding success may be evaluated (Sánchez-Mora et al., 2017). However, nowadays there are many biotechnological techniques that advance this process (Töpfer et al., 2011).

The genetic variability of a grape germplasm bank can only be effectively used when properly evaluated and quantified, and describing the inputs or genotypes is required for maintaining and characterizing both (Vanderborght 1988). Characterizing and evaluating germplasm, as well as providing better knowledge of available assessments, is essential for a more intense usage in later phases, allowing the identification of duplicate genotypes and simplifying subsequent work. In a genebank, it is possible to establish a core collection, which by definition covers with minimal redundancy the genetic diversity in cultivated and related wild species, besides allowing the identification of reproduction modes prevailing in individual genotypes (Gama et al., 2013).

In the scenario of breeding, conservation of genetic resources and the preservation of genetic diversity, germplasm characterization for immediate or future use is a very important approach that makes it possible to identify desirable developmental characteristics that increase productivity and resistance of the cultivar to the main pathogens affecting the culture. The choice of germplasm is a fundamental and crucial element in any plant breeding program, whether for the development of cultivars to be used in hybrids or for basic studies, which may significantly impact on the success or failure of the selection process (Wan et al., 2015).

The plant phenology that allows characterizing the duration of vine development phase concerning climate, especially with seasonal variations, may also be used to interpret how the different climatic regions influence the culture and are relevant for the genetic breeding programs (Leão et al., 2011). Moreover, this evaluation is also relevant since the climate changes are affecting vineyard in many parts of the world (Van Leeuwen and Darriet, 2015).

Multivariate data analysis, as opposed to other univariate statistical methods, consider multiple variables at the same time, revealing the important components through multiple interference and interactions used in scientific works as a tool allowing the classification and characterization of the genetic material (Leão et al., 2011; Borges et al., 2010; Cozzolino et al., 2009).

Many grape breeding programs search for climatic adaptation because that is very correlated with diseases attack and fruit quality. Considering the importance of knowing the grapes phenotypic divergence, the objective of this paper is to classify the productive cycle of 110 phenotypes for genetic breeding. Studying plant phases made it possible to cross different genotypes, breeding new varieties and understanding the better period to plant some cultivars. Furthermore, when knowing the number of days to harvest the grape enables cultivated new varieties in different regions.

Results and discussion

Correlation analysis

Correlations between the variables are shown in Table 1. The sprouting phase (S) was significantly correlated with flowering (F), because the initial sprouting produced branches, leaves, and flowers, but the differences between the vines in the vineyard can be decreased or increased by the weather, in particular by temperature and precipitation at the moment of budding (Lebon et al., 2005).

Under normal temperate climates and good growing conditions, the starting sprouting time and inflorescence development rate depend on the bud position on the branch in the winter, some flowers per inflorescence and the genetic potential (Vasconcelos et al., 2009).

In order to develop branches and flowers, the plant needs to overcome bud dormancy, and the latency of the gem of the vines is genetically controlled and naturally induced by photoperiod and low temperatures (Barros et al., 2007). In the State of São Paulo, as well as in other cities of Brazil and worldwide, the practice of applying plant regulator on the vine to overcome dormancy after pruning is usual to make possible the cultivation of different species of *Vitis* spp. in conditions where the climate does not properly reach cold temperatures (George et al., 1988; Or et al., 2000; Or et al., 2002; Halaly et al., 2008; Arora et al., 2003).

The sprouting and flowing phases are not correlated with the starting maturation (SM) and maturation (M), but the starting maturation was correlated with the maturation (Table 1 and Fig 1).

The start maturation and maturation are important phases for grapes, because of increasing in grape size, pH, glucose, fructose, anthocyanins and flavour compound contents and decreases tartrate and tannins (Coombe 2001). Furthermore, this two phases involved control of the timing of ripening, berry size, coloration, acidity and the relative assortment of volatile and non-volatile aroma and flavour compounds, fundamental in table and wine grape cultivars (Conde et al., 2007).

Many factors can influence the point at which maturation starts, but some authors describe the importance of climactic and genetic factors (Agasse et al., 2007; Coombe 2001; Conde et al., 2007). The genetic factors are associated with a breeding to provide new adapted cultivars or to choose genotypes with interesting characteristics for grape-producing regions (Agasse et al., 2007). Moreover, the climate factor is related to water availability in the soil, because the volumetric growth of fruit is primarily the result of water accumulation; hence, the maintenance of fruit growth requires coordination between water and solute transport, both through the vascular tissue and at the individual cells level (Leblon et al., 2005, Taiz and Zaiger 2009).

Principal component analysis and UPGMA

According to the principal component analysis (PCA), the first two principal components accounted for 81% (Table 2) of the variation in genotypes, a condition that allows analyzing divergence through graphic dispersion (Cerqueira-Silva et al., 2009). Also, regarding the productive cycle, it was possible to classify the genotypes into three groups, early, median and late (Fig 1).

The first component was associated with the starting maturation (SM) and maturation (M) phases. The second component was associated with the sprouting (S) and flowering (F) variable (Table 2).

According to Castelli and Pisani (1985), the sprouting and flowering process apparently varies among varieties of grapes, but the last two phenological periods, starting maturation and maturation, are highly correlated, with a greater influence on the classification of genotypes regarding the number of days taken to complete the productive cycle (Table 1 and Fig 1). Thus, the genotypes might be divided into early, median and late cycle. The varieties that showed the lowest starting maturation also showed low values for maturation, and the interval between these two phenological phases is small when compared with the preceding phases, flowing and sprouting.

Between the starting maturation and maturation phases, the grape berries undergo metabolic and physiological changes, and the range of these phenologic phases covers the period from early colour change (for red grapes), or translucency of the berries (for white grapes) until the harvest, ranging from 30 to 70 days, depending on the variety, rootstock, and region of cultivation (Abe et al., 2007).

Within the group classified as early cycle are 25 genotypes of hybrid species, *Vitis vinifera* and *Vitis labrusca* (Fig 1). These plants showed lower phases starting maturation and maturation and completed the cycle between 112 and 132 days. Among the best-known varieties of this group are BRS Linda, BRS Morena, Einset Seedless, Muska, Kioho I., Venus, Maria, Mars, IAC Aurora, Moscatel Azul, Moscatel Suiça,

Table 1. Correlations between four phenological phases of 110 grape phenotypes in four year cycles.

Phases	Sprouting	Flowering	Starting maturation	Maturation
Sprouting	1	0.44*	0.20	-0.01
Flowering		1	0.01	0.26
Starting maturation			1	0.78*
Maturation				1

*diference significant at $p \leq 0.01$.

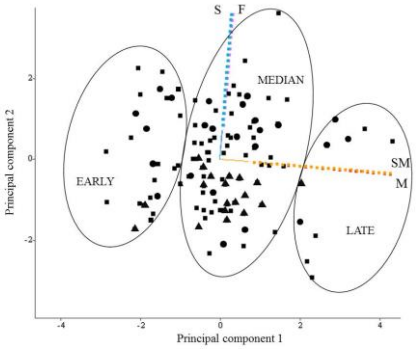

Fig 1. Principal components analysis showing the relation between the two main components and the four phenological phases, sprouting, flowering, starting maturation and maturation, divided the phenotypes about them productive cycle in three groups, early, median and late cycles. Types of grapes: Hybrid (■), *Vitis vinífera* (●) and *Vitis labrusca* (▲). (S) sprouting, (F) flowering, (SM) starting maturation and (M) maturation.

Table 2. Estimates of eigenvalues and eigenvectors associated with the principal components analysis, along with their relative and accumulated importance, relative to four phenological phases valued at 110 grape phenotypes in four cycles.

Components	Eigenvectors				Eigenvalues	Difference	Proportion (%)	Accumulated (%)
	M	SM	F	S				
1	0.705	0.705	0.062	0.053	1.79	0.34	45	45
2	-0.059	-0.056	0.704	0.705	1.44	0.88	36	81
3	-0.349	0.044	-0.706	0.706	0.56	0.34	14	95
4	0.706	-0.706	-0.038	0.041	0.22		5	100

Note: (S) sprouting, (F) flowering, (SM) starting maturation and (M) maturation.

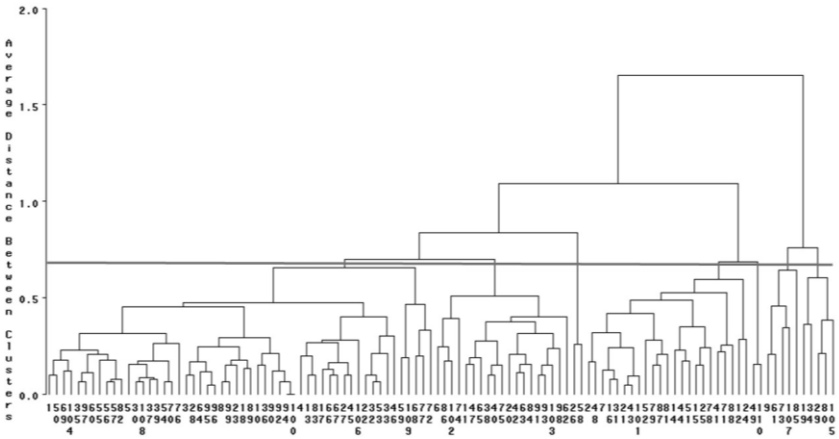

Fig 2. UPGMA tree of 110 grapes phenotypes using four phenological periods, sprouting, flowering, starting maturation and maturation, with the cut was made on the shaft at a distance of approximately 0.7, classifying them into three groups about the number of days to complete the cycle. Note: (1) Alphonse Lavalee, (2) Alwood , (3) Ananaz, (4) Arizul L, (5) Armênia, (6) Armênia I70060, (7) Athens, (8) August Giant , (9) Barrileta, (10) Beni-Fuji, (11) Benitaka, (12) Black Corintho, (13) Black Price, (14) BRS Linda, (15) BRS Morena, (16) Buffalo, (17) Carman, (18) Diamond, (19) Dona Zilá, (20) Dutchess, (21) Einset seedless, (22) Favorita, (23) Fern Munson, (24) Fiesta, (25) Flame seedless, (26) Fogarina, (27) Folha de Figo, (28) Golden Muscat, (29) Golden Queen, (30) H 4.49.69, (31) Hartford, (32) Hidalgo, (33) Highland, (34) Hubard, (35) IAC 1595-16, (36) IAC 0031-01, (37) IAC 0324-20, (38) IAC 0388, (39) IAC 0433-06, (40) IAC 0501-06, (41) IAC 0506-33, (42) IAC 0514-06, (43) IAC 0775-26, (44) IAC 0842-04, (45) IAC 0871, (46) IAC 0871-05 (47) IAC 0871-41, (48) IAC 1298-21, (49) IAC 1398-21, (50) IAC 1410-08, (52) IAC 1596-02, (53) IAC 1726-03, (54) IAC Juliana, (55) Igawa 0668, (56) Igawa 0682, (57) Igawa 1010, (58) Igawa 1011, (59) Igawa 1012, (60) Igawa 1015, (61) Iowa, (62) Isabel Sport, (63) Itália, (64) Italiana, (65) J 7602-66, (66) J 7604-15, (67) J7602-44, (68) Japonesa, (70) Kioho BG, (71) Kioho H, (71) Kioho I, (72) Lakemont seedless, (73) Líbano, (74) Liberty, (75) Lomanto, (76) Lubeck, (77) Lucile, (78) Maria, (79) Mars, (80) Moscatel Aragentina, (81) Moscatel Azul, (82) Moscatel de Hamburgo, (83) Moscatel de Oieiras, (84) Moscatel Nazareno, (85) Moscatel Rosada Portuguesa, (86) Moscatel Rosado, (87) Moscatel Suíça, (88) Muska, (89) Niabell, (90) Niagara, (91) Niagara Branca Gigante, (92) Niagara Branca Oval, (93) Niagara Maravilha, (94) Niagara Rajada, (95) Niagara Rosada, (96) Niagara Rosada Escura, (97) Niagara Rosada Gigante, (98) Niagara Rosada Variegada, (99) Niagara seedless, (100) Niagara Steck, (101) Perla de Csaba, (102) Pirovana 54, (103) Portugais bleu, (104) Romana, (105) Rosaki, (106) Ruby seedless, (107) Tardia de Caxias, (108) Tieta, (109) Topaz e (110) Venus.

Table 3. Grapes phenotypes classified by species and identification number.

Number	Phenotype	Species	Number	Phenotype	Species
1	Alphonse Lavallee	*Vitis vinifera*	56	Igawa 0682	Hybrid
2	Alwood	*Vitis labrusca*	57	Igawa 1010	Hybrid
3	Ananaz	Hybrid	58	Igawa 1011	Hybrid
4	Arizul L	*Vitis vinifera*	59	Igawa 1012	Hybrid
5	Armênia	*Vitis vinifera*	60	Igawa 1015	Hybrid
6	Armênia I70060	*Vitis vinifera*	61	Iona	Hybrid
7	Athens	Hybrid	62	Isabel Sport	*Vitis labrusca*
8	August Giant	Hybrid	63	Itália	*Vitis vinifera*
9	Barrileta	Hybrid	64	Italiana	*Vitis vinifera*
10	Beni-Fuji	*Vitis vinifera*	65	J 7602-66	Hybrid
11	Benitaka	*Vitis vinifera*	66	J 7604-15	Hybrid
12	Black Corintho	*Vitis vinifera*	67	J7602-44	Hybrid
13	Black Price	*Vitis vinifera*	68	Japonesa	Hybrid
14	BRS Linda	Hybrid	69	Kioho BG	Hybrid
15	BRS Morena	Hybrid	70	Kioho H	Hybrid
16	Buffalo	Vitis labrusca	71	Kioho I	Hybrid
17	Carman	Hybrid	72	Lakemont seedless	Hybrid
18	Diamond	*Vitis Labrusca*	73	Líbano	Hybrid
19	Dona Zilá	Hybrid	74	Liberty	Hybrid
20	Dutchess	Vitis labrusca	75	Lomanto	Hybrid
21	Einset seedless	Hybrid	76	Lubeck	Hybrid
22	Favorita	Hybrid	77	Lucile	Hybrid
23	Fern Munson	*Vitis vinifera*	78	Maria	Hybrid
24	Fiesta	*Vitis vinifera*	79	Mars	Hybrid
25	Flame seedless	*Vitis vinifera*	80	Moscatel Argentina	*Vitis vinifera*
26	Fogarina	Hybrid	81	Moscatel Azul	*Vitis vinifera*
27	Folha de Figo	*Vitis labrusca*	82	Moscatel de Hamburgo	*Vitis vinifera*
28	Golden Muscat	*Vitis vinifera*	83	Moscatel de Oieiras	*Vitis vinifera*
29	Golden Queen	*Vitis vinifera*	84	Moscatel Nazareno	*Vitis vinifera*
30	H 4.49.69	*Vitis vinifera*	85	Moscatel Rosada Portuguesa	*Vitis vinifera*
31	Hartford	Hybrid	86	Moscatel Rosado	*Vitis vinifera*
32	Hidalgo	Hybrid	87	Moscatel Suíça	*Vitis vinifera*
33	Highland	Hybrid	88	Muska	Hybrid
34	Hubard	Hybrid	89	Niabell	*Vitis labrusca*
35	IAC 1595-16	Hybrid	90	Niagara	*Vitis labrusca*
36	IAC 0031-01	Hybrid	91	Niagara Branca gigante	*Vitis labrusca*
37	IAC 0324-20	Hybrid	92	Niagara Branca Oval	*Vitis labrusca*
38	IAC 0388	Hybrid	93	Niagara Maravilha	*Vitis labrusca*
39	IAC 0433-06	Hybrid	94	Niagara Rajada	*Vitis labrusca*
40	IAC 0501-06	Hybrid	95	Niagara Rosada	*Vitis labrusca*
41	IAC 0506-33	Hybrid	96	Niagara Rosada Escura	*Vitis labrusca*
42	IAC 0514-06	Hybrid	97	Niagara Rosada Gigante	*Vitis labrusca*
43	IAC 0775-26	Hybrid	98	Niagara Rosada variegada	*Vitis labrusca*
44	IAC 0842-04	Hybrid	99	Niagara seedless	*Vitis labrusca*
45	IAC 0871	Hybrid	100	Niagara Steck	*Vitis labrusca*
46	IAC 0871-05	Hybrid	101	Perla de Csaba	*Vitis vinifera*
47	IAC 0871-41	Hybrid	102	Pirovano 54	*Vitis vinifera*
48	IAC 0871-41	Hybrid	103	Portugais bleu	*Vitis vinifera*
49	IAC 1398-21	Hybrid	104	Romana	*Vitis vinifera*
50	IAC 1410-08	Hybrid	105	Rosaki	*Vitis vinifera*
51	IAC 1595-02	Hybrid	106	Ruby seedless	*Vitis vinifera*
52	IAC 1596-02	Hybrid	107	Tardia de Caxias	Híbrida
53	IAC 1726-03	Hybrid	108	Tieta	Hybrid
54	IAC Juliana	Hybrid	109	Topaz	Hybrid
55	Igawa 0668	Hybrid	110	Venus	Hybrid

Perla de Csaba, Flame Seedless, Black Corinth, Fiesta, Alwood and Buffalo (Fig 2). The varieties Venus and Mars are American hybrids with early cycles (Camargo 1993; Botelho et al., 2002; Tecchio et al., 2009). Corroborating Borges et al., (2010) grouped into Semi-Arid Brazilian conditions the varieties, Flame Seedless and Fiesta, with 59% similarity in days to complete the cycle.

The values to reach the starting maturation and maturation phases concentrated near the central axis the principal components graphic, with the number of days to harvest ranging from 134 to 156, classified as corresponding to the median cycle, including 75 genotypes of all species (Fig 1). Among the main ones are the varieties Arizul L., Beni Fugi, Diamond, Fogarina, Folha de figo (fig leaf), Hidalgo, IAC Juliana, Itália, Italiana, Kiono B.G., Lakemont seedless, Liberty, Lubeck, Moscato Hamburgo, Moscato Rosado, Niagara Branca, Niagara Rosada, Niagara Rajada, Niagara seedless, Portugal, Romana, Tieta and Topaz (Fig 2).

In Turkey, the cultivation of *Vitis labrusca*, Niagara Branca presented a median cycle of approximately 148 days from budding to harvest (Köse 2014), and in Rio Grande do Sul, Brazil, the cultivation of Niagara Rosada and Niagara Branca also presented a white median cycle of approximately 147 days of budding to harvest (Anzanello et al., 2012).

The 10 genotypes classified as late cycle presented the highest values to reach the starting maturation and maturation phases, ranging from 156 to 174 days to complete the cycle (Fig 1). Among them are Barrilete, Dona Zilá, Golden Queen, Highland, Iona, Líbano, Moscato Argentina, Moscato Rosada Portuguesa, Rosaki Rosada and Tardia de Caxias varieties (Fig 2). According to Shiraishi (2000), the Rosaki variety features a longer cycle when compared with Buffalo, an early cycle variety. Corroborating, the genotypes Dona Zilá and Tardia de Caxias feature a longer cycle of 20 more days to harvest compared with the Niagara Rosada, a median cycle variety (Camargo et al., 1994).

These results can be better viewed on the dendrogram graphic (Figure 2), where a group comprised of the same varieties, having a cut on the shaft at a distance of approximately 0.7, classifying them into three groups regarding the number of days to complete the cycle. It can be observed that the shortest distance between genotypes was 0, between Niagara Rajada and Niagara Steck varieties, two median cycle cultivars that are both somatic mutations of the Niagara Branca variety (Souza 1959), and the greatest distance was 0.3604 between Dona Zilá and Highland varieties, classified as a late cycle.

Materials and methods

Experimental area and plant material

The active collection of germplasm of *Vitis* spp located in the Agronomic-IAC, Brazil, situated at 23°06'' South, 46°56'' West, at an altitude of 745 m, with pluvial precipitation of 1400 mm, 19.5 °C temperature, and 70.6% yearly median relative humidity. The climate is classified as Cwa, and the soil as Dystrophic Cambisols.

We analyzed the phenological behaviour of 110 genotypes of grape, commercial and non-commercial varieties belonging to the species *Vitis vinifera*, *Vitis labrusca* and hybrids among them, during production 4 years, from 2012 to 2015, to classify them regarding the productive cycle of the varieties presented in the germplasm grape collection; these are identified by the numbers 1 to 110 (Table 3).

The vines were grafted onto rootstock IAC 766 'Campinas', conducted in espalier and spaced in 2.0 m between lines and 1.0 m between plants. Each genotype consisted of three plants pruned every year during the month August, leaving one or two gems per branch. Hydrogenated cyanamide (H_2CN_2 Dormex, 49%) was applied by targeted spraying at a concentration of 5% to standardize the sprouting,

Conduction of experiment

Duration of days was evaluated after pruning of the main vine phenologic phases, using the scale proposed by Eichhorn and Lorenz (1984). Two evaluations were performed a week until flowering and, after that phase, such evaluations were once a week. From visual observations of the phenologic phases, the period between pruning and the beginning of sprouting, full flowering with 50% flowers opened, beginning of maturation (*veraison*) and maturation (harvest), visually mature fruits and content of soluble solids above 14ºBrix.

Statistical analysis

Statistical analysis was performed using the SAS (Statistical Analysis Software, version 9.2) system. Multivariate analyses, such as the correlation between the variables ($p < 0.01$), principal component analysis (PCA) and the UPGMA clustering method were obtained from dissimilarity matrix computed by Euclidean distance and used to classify the genotypes per the productive cycle. The minimum criteria absorption on 80% of total variation was used in the first components (Cruz and Regazzi, 1994).

Conclusion

The starting maturation and maturation are the most divergent periods between the grapes phenotypes. The PCA jointly with the group by the UPGMA method classified the phenotypes according to three different productive cycles. In that way, 25 genotypes were classified as early, 75 as median and 10 as late. The grapes phenotypes evaluated had good variability regarding the productive cycle.

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