DOI: http://dx.doi.org/10.1590/1678-992X-2017-0387

ISSN 1678-992X

SCIENTIA AGRICOLA

Resistance to *Pratylenchus brachyurus* in Vitis species population through multivariate approaches and mixed models

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Edited by: Leonardo Oliveira Medici

Received December 07, 2017
Accepted May 18, 2018

ABSTRACT: Genetically diverse interspecific hybrids of *Vitis* were selected for resistance to *Pratylenchus brachyurus*. Three segregating populations with 57 hybrid crosses were evaluated. The parents included *Vitis romanetii*C166-043 × 07355-075, 06354-047 × Cereza and 06354-047 × Nocera, selected from the germplasm bank at the University of California, Davis, the United States. The experiment was arranged in a randomized block design, with three replications and three plants per plot. Root mass, nematodes per gram of root and reproduction factor were determined and used as quantitative variables; 16 multi categoric descriptors were also evaluated. The traits were analyzed using the Ward-Modified Location Model procedure (Ward-MLM) for the composition of genotype groups. Genetic parameters and prediction of genetic values by Restricted Maximum Likelihood / Best Linear Unbiased Prediction (REML / BLUP) were assessed. The Ward-MLM classification strategy supported the formation of three homogeneous groups. Group I comprised 13 hybrids; Group II, 26 hybrids; and Group III, 18 hybrids. Groups II and III contained hybrids resistant to *P. brachyurus*. High broad sense heritability values were found for root mass, reproduction factor and nematodes per gram of root, which provided genetic gain and allowed selection of resistant genotypes available for cloning, since the total genetic variance occurred due to the dominance effects. Of the 57 genotypes assessed, those with the lowest genotypic values for reproduction factor were selected as resistant, including: CH3.2, CH3.23, CH3.8, CH3.37, CH3.38, CH3.41, CH3.36, CH2.1, CH2.7, CH1.1, CH1.3 and CH1.2.
Keywords: *Vitis* genotypes, nematode, root, genetic variability

Introduction

In the international scenario, the Brazilian viticulture occupied the 11thplace in grape production and the 13th in wine production in 2015, accounting for a total production of 1.6 million tons of grapes in 2016 (FAO, 2016). Therefore, viticulture in Brazil has huge social and economic importance for generating employment and income, considering both table grapes and wine production (IBGE, 2016).

However, nematodes seriously impair grapevine establishment by hindering development, decreasing vigor and greatly reducing the plant root system, thereby decreasing fruit yield and quality. It is estimated that nematode feeding reduces annual grape production by 20 to 25 % worldwide (Puerari et al., 2012). The specie *Pratylenchus brachyurus* stands out for its wide dissemination in regions of agriculture cultivation. It has been recognized as one of the worst soil-borne pests of grapevines, especially under high populations in the soil (Téliz et al., 2007; Ferris et al., 2012).

The increase in population levels and damage caused by *P. brachyurus* represent a serious problem for production systems, compromising tropical viticulture (Puerari et al., 2012). The application of nematicides as a control method is an expensive and inefficient practice. The use of resistant genotypes is the main strategy for nematodes control in the vine, as it does not increase production costs, nor does it harm the environment. Therefore, the selection of resistant hybrids is necessary to be used as rootstocks for good yield in viticulture (Walker et al., 1994; Zasada et al., 2012).

The inclusion of wild species in breeding programs is one of the key approaches to pest and disease resistance breeding, since these species may have resistance genes not found in cultivated plants. Therefore, knowing the genetic variability to select resistant clones in segregating populations is particularly important for breeders when the level of variation in breeding populations is not fully known or explored (Walker et al., 2010; Viana et al., 2011).

This study aimed to characterize populations of interspecific crosses of *Vitis* based on qualitative and quantitative descriptors and estimate genetic parameters capable of selecting interspecific hybrids resistant to *P. brachyurus* for grapevine breeding programs.

Materials and Methods

Populations assessed

Three segregating populations with 57 hybrids obtained from crosses involving *Vitis* spp. were investigated in relation to their resistance to *P. brachyurus* (Table 1).

Experimental details and phenotyping

Cuttings of hybrids were rooted and then planted in 7 L pots containing a soil: sand mixture atratio2:1 (v/v) and kept in a greenhouse in the municipality of Campos dos Goytacazes, Rio de Janeiro, Brazil (21°44′ S, 41°19′

Table 1 – Parents and crosses used in the morphological characterization and evaluation for resistance to *P. brachyurus* at the University of California, Davis, USA.

Parents[a]	Traits
07355-075 (*V. vinifera*)	Advanced wine grape selection with resistance to Pierce's disease originating from *V. arizonica*
Vitis romanetii C166-043 (*V. romanetii*)	Chinese species, DVIT2732, from the National Clonal Germplasm Repository - Davis
Nocera (*V. vinifera*)	An Italian wine grape from Sicily
Cereza (*V. vinifera*)	Large berried Italian table grape
06354-047 (*V. vinifera/M. rotundifolia*)	Powdery mildew resistant wine grape selection with resistance from a *V. vinifera* × *Muscadinia rotundifolia* hybrid
Crosses	Number of hybrids assessed
Vitis romanetii C166-043 × 07355-075	7 - CH1[a]
06354-047 ×Cereza	9 - CH2[a]
06354-047 ×Nocera	41 - CH3[a]
Total	57

[a]CH1, CH2 and CH3 = hybrid obtained from crossed 1, 2 and 3 respectively.

W, altitude 12 m), with average temperature and relative humidity of 31.3 °C and 83 %, respectively. Meteorological data were obtained from a thermo-hygrometer inside the greenhouse.

The experiment was arranged in a randomized block design, with three replications and three plants per plot of 57 interspecific *Vitis* hybrids, and a maize cultivar, 'BR 106', which was used as source of inoculum and as a susceptible control to verify the viability of nematode inoculum. Maize is very sensitive to *P. brachyurus* infestation and cultivar 'BR 106' was used in this work for its wide distribution in commercial crops throughout Brazil.

After four months (in Nov 2015), the plants were inoculated with a suspension of 800 adults and juveniles of *P. brachyurus*. The nematode suspension was calibrated to 400 nematodes mL^{-1} and placed into two evenly distributed holes around the grape and maize seedlings.

Maize was evaluated approximately 90 days after inoculation for nematode extraction. After that, each pot received a new plant to be evaluated in 180 days for grapes and a second 90 days for maize again. The root systems of grape and maize plants were collected for nematode extraction. Nematodes were extracted from the roots using the methodology proposed by Coolen and D'Herde (1972). The samples obtained were evaluated and all the specimens of *P. brachyurus*in each sample were counted under a stereoscopic microscope, using the Peters's counting slide.

From the evaluations, we determined the root fresh mass, nematodes per gram of root and the reproduction factor ($RF = Pf / Pi$, where FR, Pf and Pi refer to the reproduction factor, final population and initial population, respectively). The plants were classified as immune ($FR = 0$), resistant ($0 < FR < 1$) and moderately resistant or susceptible ($FR > 1$), as described by Oostenbrink (1966). Fresh root mass was determined from the plants were carefully removed to the pots and the separate aerial part of the root system. The root systems were washed in running water, the roots were placed on absorbent paper until water excess was removed, then they were weighed and fresh root mass was obtained.

The interspecific hybrids were also characterized by morphological descriptors. Sixteen qualitative descriptors were used (vegetative stage) along with three quantitative characters related to *P. brachyurus* resistance, for 19 descriptors (Table 2). Ampelographic characters were described by using OIV descriptors (OIV, 2013) with small modifications. Sixteen parameters were observed and most of them were measured in different organs of the adult plant (Table 2). These characters were observed ten days after inoculations in plants of each plot (three plants), where five young and adult leaves were measured per plant and the statistic mode was calculated for each plant.

Ward-MLM multivariate analysis

The quantitative and qualitative variables were analyzed simultaneously, using the Ward-MLM procedure to compose genotype groups, using the procedure CLUSTER and IML in SAS program (Statistical Analysis System, version 9.2). The distance matrix was obtained by the Gower algorithm (Gower, 1971) using the Ward grouping method. The ideal number of groups was defined according to the pseudo-F and pseudo-t^2 criteria, combined with the likelihood profile and the likelihood ratio test.

The Gower dissimilarity index was used because the set of variables in the study form a mixed group of qualitative and quantitative traits in which a single index of dissimilarity is generated, ranging from 0 to 1. Finally, a complete MLM analysis was carried out for the number of groups (g) defined, which described the classification results on a table presenting the groups formed, while a canonical analysis was used for the quantitative variables. To obtain the canonical variables, the CANDISC procedure in SAS program was used, which contained the canonical coordinates for observations (Crossa and Franco, 2004).

Estimates of genetic parameters and individual selection using the REML/BLUP procedure

To select the representative value of a clone, genotypic values for each quantitative trait were obtained

Table 2 – Descriptors used for the characterization and assessment of a population from the interspecific cross in *Vitis* spp. UENF, Campos dos Goytacazes, Rio de Janeiro, Brazil, 2016

Descriptors	Description
Qualitative	
Young branch: opening in the tip;	1 = closed; 3 = semi-open; 5 = fully open
Young branch: Anthocyanin pigmentation in the tip;	1 = absent or very weak; 3 = weak; 5 = medium; 7 = strong; 9 = very strong
Young leaf: color of the upper limb face;	1 = yellow-green; 2 = green with anthocyanic areas; 3 = clear red-copper; 4 = dark red-copper; 5 = wine-red
Adult leaf: limbsize;	1 = very small; 3 = small; 5 = medium; 7 = large; 9 = very large
Adult leaf: limbshape;	1 = cordiform; 2 = deltoid; 3 = pentagonal; 4 = orbicular; 5 = reniform
Adult leaf: number of lobes;	1 = none; 2 = three; 3 = five; 4 = seven; 5 = above seven
Adult leaf: depth of the upper lateral sinuses;	3 = shallow; 5 = medium; 7= deep
Adult leaf: arrangement of the lobes of the upper lateral sinuses;	1 = open; 2 = closed; 3 = slightly overlapped; 4 = very overlapped
Adult leaf: shape of the basis of the petiolar sinus;	1= concave (in "u"); 2 = straight (in "v"); 3 = convex
Adult leaf: arrangement of the lobes of the petiolar sinus;	1 = fully open; 2 = very open; 3 = half open; 4 = little open; 5 = closed; 6 = slightly overlapped; 7 = half overlapped; 8 = very overlapped; 9 = fully overlapped
Adult leaf: length of teeth;	3 = short; 5 = medium; 7 = long
Adult leaf: teeth length / width ratio;	1 = very small; 3 = small 5 = medium; 7 = large; 9 = very large
Adult leaf: teethshape	1 = both sides are concave; 2 = both sides are rectilinear; 3 = both sides are convex; 4 = one side is concave and the other is convex; 5 = combination of both sides are rectilinear with both sides are convex
Adult leaf: anthocyanin pigmentation of major veins on upper limb face	1 = absent or very weak; 3 = weak; 5 = medium; 7 = strong; 9 = very strong
Adult leaf: length of petiole in relation to central vein;	4 = shorter; 5 = equal; 6 = longer
Adult leaf: density of erect hairs on the petiole	1 = absent or very low; 3 = low; 5 = medium; 7 = high; 9 = very high
Quantitative	
Root Mass (g)	Total mass root system of plants 180 days after inoculation
Reproduction Factor	Ratio between final population and initial population of nematodes
Nematodes per gram of root	10 g of roots were weighed and separated and chopped to determine nematode numbers

using the REML (Restricted Maximum Likelihood) procedure and applied to the mixed linear model in its matrix form to evaluate related clones in randomized block designs with several plants per plot: $Y = Xr + Za + Zd + Wp + e$. Where: Y is the data vector; r= is the vector of repetition effects (assumed to be fixed) added to the general average; a= is the vector of the additive genetic effects (assumed to be random); d= is the vector of the dominance genetic effects (assumed to be random); p = is the vector of plot effects (assumed to be random); e = is the vector of errors or residuals (assumed to be random). Capital letters refer to incidence matrices for these effects.

The following genetic parameters were estimated: additive genetic variance (σ_a^2), dominance genetic variance (σ_d^2), environmental variance between plots (σ_{parc}^2), residual (environmental) variance (σ_e^2), individual phenotypic variance (σ_f^2), narrow sense individual heritability ($\hat{h}_a^2$), individual heritability of dominance effects ($\hat{h}_d^2$), broad sense individual heritability ($\hat{h}_g^2$), coefficient of determination of plot effects (c_{parc}^2) and overall average of the experiment.

The structures of averages and variances are given by:

$$E\begin{bmatrix} y \\ a \\ d \\ c \\ e \end{bmatrix} = \begin{bmatrix} Xb \\ 0 \\ 0 \\ 0 \\ 0 \end{bmatrix};$$

$$Var\begin{bmatrix} y \\ a \\ d \\ c \\ e \end{bmatrix} = \begin{bmatrix} V & ZA\sigma_a^2 & ZD\sigma_D^2 & WI\sigma_c^2 & I\sigma_e^2 \\ A\sigma_a^2 Z' & A\sigma_a^2 & 0 & 0 & 0 \\ D\sigma_d^2 Z' & 0 & D\sigma_d^2 & 0 & 0 \\ I\sigma_a^2 W' & 0 & 0 & I\sigma_c^2 & 0 \\ I\sigma_a^2 & 0 & 0 & 0 & I\sigma_e^2 \end{bmatrix}$$

where: $V = ZA\sigma_a^2 Z' + ZD\sigma_d^2 Z' + WI\sigma_c^2 + I\sigma_e^2$

Mixed Model Equations:

$$\begin{bmatrix} X'X & X'Z & X'Z & X'W \\ Z'X & Z'Z + A^{-1}\lambda_2 & Z'Z & Z'W \\ Z'X & Z'Z & Z'Z + D^{-1}\lambda_2 & Z'W \\ W'X & W'Z & W'Z & W'W + I\lambda_3 \end{bmatrix} \begin{bmatrix} \hat{b} \\ \hat{a} \\ \hat{d} \\ \hat{c} \end{bmatrix} = \begin{bmatrix} X'y \\ Z'y \\ Z'y \\ W'y \end{bmatrix}$$

where:

$$\lambda_1 = \frac{\sigma_e^2}{\sigma_a^2} = \frac{1 - h_e^2 - c^2}{h^2};$$

$$\lambda_2 = \frac{\sigma_e^2}{\sigma_d^2} = \frac{1 - h_a^2 - c^2}{h_a^2 - h^2};$$

$$\lambda_3 = \frac{\sigma_e^2}{\sigma_c^2} = \frac{1 - h_e^2 - c^2}{c^2}$$

σ_d^2 and h_a^2: Genetic variance of dominance and broad sense heritability, respectively; D: matrix of genetic correlation of dominance between the individuals assessed.

The system provided predictions of the additive ($\hat{a}$) and dominance ($\hat{d}$) effects isolatedly. The total genotypic values, given by $\hat{g} = \hat{a} + \hat{d}$, can be predicted directly from the equations of mixed models:

$$\begin{bmatrix} X'X & X'Z & X'W \\ Z'X & Z'Z + G^{-1}\sigma_E^2 & Z'Z \\ W'X & W'Z & W'W + I\lambda_3 \end{bmatrix} = \begin{bmatrix} \hat{b} \\ \hat{g} \\ \hat{c} \end{bmatrix} = \begin{bmatrix} X'y \\ Z'y \\ W'y \end{bmatrix},$$

where: $G = \sigma_a^2 + D\sigma_d^2$

Iterative estimators of variance components by REML via EM algorithm:

$$\hat{\sigma}_e^2 = \left[y'y - \hat{b}'X'y = \hat{a}'Z'y - \hat{d}'Z'y - \hat{c}'W'y \right] / \left[N - r(x) \right]$$

$$\hat{\sigma}_c^2 = \left[\hat{c}'\hat{c} + \hat{\sigma}_e^2 tr C^{44} \right] / s ;$$

$$\hat{\sigma}_a^2 = \left[a'A^{-1} - \hat{a}' + + \hat{\sigma}_e^2 tr(A^{-1}C^{22}) \right] / q ;$$

$$\hat{\sigma}_d^2 = \left[\hat{d}'^{D^{-1}} - \hat{d} + \hat{\sigma}_e^2 tr(D^{-1}C^{22}) \right] / q .$$

Results

Ward-MLM multivariate analysis

The Ward-MLM classification strategy, which simultaneously used quantitative traits related to resistance to *P. brachyurus* and qualitative, such as morphological and agronomic descriptors, defined the optimal number in three groups and was capable of discriminating 57 interspecific hybrids (Figure 1). The likelihood function was used to determine the ideal number of groups, with an increment value of 66.51 (Figure1). According to Barbé et al. (2010), the analysis of the likelihood function can define accurate criteria for formation of groups, resulting in less subjective groups.

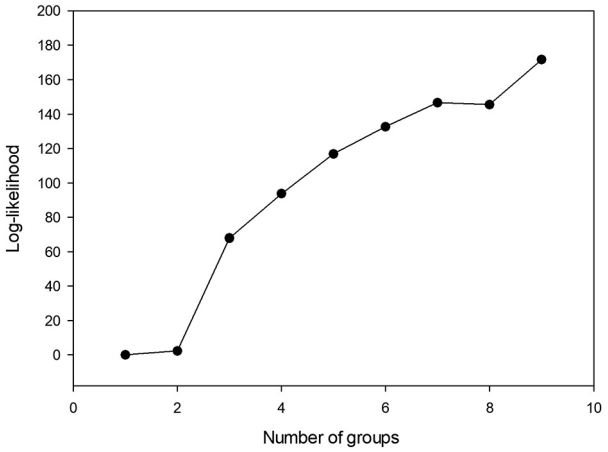

Figure 1 – Logarithmic probability function (Log-likelihood) for the number of groups formed by Ward-Modified Location Model procedure (Ward-MLM) strategy in interspecific hybrids of Vitis spp. UENF, Campos dos Goytacazes, Rio de Janeiro, Brazil, 2016.

The traits of young shoot tips showed that most hybrids presented opening of shoot tips, with predominance of hybrids presenting absent and weak anthocyanic pigmentation (70 %). Only group II contained four hybrids with strong anthocyanin pigmentation (Table 3).

Considering the traits evaluated in the adult leaves, hybrids of group I presented between zero and three lobes, whereas hybrids of groups II and III had predominantly between three and five lobes (91 %). Limb-shaped leaves were predominantly adeltoid (73 %) with upper lateral sinus ranging from shallow to deep. For the base shape of the petiole sinuses, most hybrids in each group presented leaves of the rectilinear type. Hybrids of groups I and II had leaflet size ranging from small to very large, while group III allocated hybrids with predominantly small leaf limbs (13 hybrids) (Table 3).

For the traits evaluated in adult leaves, hybrids had zero to five lobes, adeltoid-shaped leaves, shallow-to-deep superior lateral sinuses, petiolar sinuses ranging from concave, convex to rectiliniear, and limb sizes ranging from very small to very large (Table 3).

Regarding the variables related to resistance to *P. brachyurus* for the susceptible control, maize 'BR 106', the average values obtained for root mass, number of nematodes per gram of root and reproduction factor were 74.87, 86.58 and 29.40, respectively, confirming viability of the inoculum used in this work (Table 6).

Wide variation in the quantitative variables related to *P. brachyurus* resistance was detected among the hybrids evaluated (Table 4). Group I had 13 genotypes with the highest values for reproduction factor and nematodes per gram of root, but lower values for root mass. The minimum value for the reproduction factor of this group was 4.09 and the number of nematodes per gram of roots was 21.74. All hybrids were susceptible to high nematode reproduction rates and reproduction factors above 1.0. The susceptible hybrids had the highest values for this trait. One hybrid in this group was from cross *V. romanetii* C166-043 × 07355-075, two from cross 06354-047 × Cereza, and ten from cross 06354-047 × Nocera.

Most genotypes (26 hybrids) were clustered in Group II. Five were obtained from cross *V. romanetii* C166-043 × 07355-075; five, from cross 06354-047 × Cereza; and 16, from cross 06354-047 × Nocera. Some resistant genotypes had reproduction factors equal to 0.7. None of the hybrids was considered highly resistant to *P. brachyurus*, given that nematodes were found in root systems of these hybrids and their reproduction factors were higher than zero (Table 4).

Group III had 18 genotypes, five were resistant with the minimum reproduction factor of 0.35. Most hybrids in this group belong to 06354-047 × Nocera population (15 hybrids) and the other hybrids, from crosses 06354-047 × Cereza (two hybrids) and *Vitis romanetii* C166-043 × 07355-075 (one hybrid). Groups II and III were the most homogeneous, with an average reproduc-

Table 3 – Traits and number of interspecific hybrids per group of multicategoric traits in each of the three groups formed by the Ward-MLM strategy. UENF, Campos dos Goytacazes, Rio de Janeiro, Brazil, 2016

Descriptors	Groups		
	G I (13)	G II (26)	G III (18)
Young branch: openness in the tip			
Closed	-	6	3
Open	13	20	15
Young branch: anthocyanin pigmentation of the tip			
Absent or weak	11	16	13
Medium	2	6	5
Strong	-	4	-
Young Leaf: color of the upper limb face			
Yellow-green	1	6	6
Green with anthocyanicareas	10	19	11
Light copperred	1	1	1
Darkcopperred	1	-	-
Winered	-	-	-
Adult leaf: limbshape			
Cordiform	3	2	2
Deltoid	8	21	13
Pentagonal	2	3	3
Orbicular	-	-	-
Reniform	-	-	-
Adult leaf: number of lobes			
None	4	-	-
Three	9	11	11
Five	-	14	7
Seven	-	-	-
Above seven	-	1	-
Adult leaf: depth of the upper lateral sinuses			
Shallow	2	5	7
Medium	6	12	7
Deep	5	9	4
Adult leaf: arrangement of the upper lateral sinuses			
Open	10	17	12
Closed	3	8	5
Very overlapped	-	1	1
Slightly overlapped	-	-	-
Adult leaf: shape of the basis of the petiolar sinus			
Concave (in “u”)	5	7	1
Rectilinear (in “v”)	6	10	10
Convex	2	9	7
Adult leaf: arrangement of the lobes of the petiolar sinuses			
Fully open	9	10	12
Half open	2	13	5
Slightly open	1	1	1
Closed	1	2	-
Overlapped	-	-	-
Adult leaf: tooth length			
Short	9	13	14
Medium	3	10	3
Long	1	3	1
Adult leaf: tooth length/tooth width ratio			
Short	2	6	3
Medium	10	17	14
Large	1	3	1
Adult leaf: size of the limb			
Verysmall	-	1	4
Small	7	13	13
Medium	3	8	1
Large	2	2	-
Verylarge	1	2	-
Adult leaf: shape of the teeth			
Both sides are concave	2	11	5
Both sides are rectilinear	3	12	9
Both sides are convex	8	1	4
One side is concave and the other is convex	-	2	-
Rectilinear sides with convex sides	-	-	-
Adult leaf: Anthocyanin pigmentation of the major veins in the upper limb			
Absentorweak	7	20	15
Medium	5	3	2
Strong	1	3	1
Adult leaf: length of the petiole in relation to the central vein			
Shorter	7	19	12
Equal	5	6	4
Longer	1	1	2
Adult leaf: Density of erect hairs on the petiole			
Absent or low	8	17	16
Medium	4	4	2
High	1	5	-

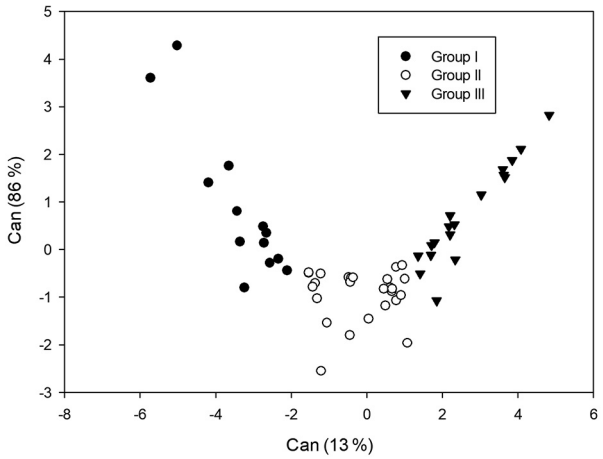

Figure 2 –The first two canonical variables for the three groups formed by the Ward-Modified Location Model procedure (Ward-MLM). UENF, Campos dos Goytacazes, Rio de Janeiro, Brazil, 2016.

tion factor value of 5.04 and 2.69, and low standard deviation estimate of 2.60 and 2.11, respectively. The most resistant genotypes with the highest estimates for root mass (100.93 and 120.0 g) and the smallest nematode populations (19.95 and 8.82), respectively, were found in these groups. Hybrids with the smallest number of nematodes per gram of root and, consequently, lower reproduction factor, also exhibited a more developed root system, a trait evaluated by root mass (Table 4).

Table 4 – Minimum and maximum values, average and standard deviation of quantitative traits for each of the three groups and coefficient of correlation of quantitative variables with the first two canonical variables (CAN). UENF, Campos dos Goytacazes Rio de Janeiro, Brazil, 2016

Variables	Parameters	Groups			CAN	
		G I (13)	G II (26)	G III (18)	CAN 1	CAN 2
Root Mass	Minimum	65.40	71.00	100.50	1.88	1.94
	Maximum	97.00	133.20	134.50		
	Average	79.80	100.93	120.0		
	Standard Deviation	9.19	15.55	10.20		
Reproduction Factor	Minimum	4.09	0.71	0.35	-1.65	-6.55
	Maximum	14.22	9.25	7.68		
	Average	8.34	5.04	2.69		
	Standard Deviation	3.38	2.60	2.11		
Nematodes per gram of root	Minimum	21.74	3.87	1.33	0.33	7.32
	Maximum	72.00	33.64	22.80		
	Average	41.37	19.25	8.82		
	Standard Deviation	14.99	8.86	6.56		

Table 5 – Distance between the groups formed by the Ward-MLM procedure (proposed by Franco et al., 1998). Data analyzed at UENF, Campos dos Goytacazes, Rio de Janeiro, Brazil, 2016

Groups	Group I	Group II	Group III
Group I	-	37.83	87.88
Group II	37.83	-	36.11
Group III	87.88	36.11	-

The first two canonical variables (CAN) obtained through the Ward-MLM methodology explained 99 % of the total variation (Figure 2). According to Crossa and Franco (2004), if the first two canonical variables result in estimates above 80 % for the total variation, a satisfactory interpretation of variability of hybrids can be obtained, which was achieved in this study. This allowed an appropriate two-dimensional graphic presentation of relationships between groups and hybrids within the groups.

Based on the first canonical variable, the traits that mostly contributed to the quantification of genetic divergence were root mass, with 1.88, and reproduction factor, -1.65 (Table 4). A separation between Groups I and III formed by the Ward-MLM procedure and approximation of Groups I and II and between Groups II and III was observed (Table 5 and Figure 2). Groups II and III include resistant hybrids with the smallest nematode populations in the roots.

During the experiment, susceptible plants presented symptomatic reactions, such as necrotic lesions of various sizes and colors in the vine roots infested with *P. brachyurus*. In addition, several symptoms were detected on shoots of susceptible plants, including loss of vigor, varying plant size, yellowing and premature leaf drop, wilt during the hottest hours of the day, slow growth, necrotic leaves and plant death due to its inability to produce an adequate root system. Such symptomatic reactions are typical of the genus *Pratylenchus* in grapevines (Zheng et al., 2010; Zasada et al., 2012).

Table 6 – Estimates of variance components and genetic parameters for variables: root mass (g), reproduction factor, and nematodes per gram of root. Data from 57 clones of *Vitis* spp. interspecific populations selected for resistance to nematode *P. brachyurus*. Data acquired and analyzed at UENF, Campos dos Goytacazes, Rio de Janeiro, Brazil, 2016

Variancecomponents (Individual REML)*	Traits		
	Root Mass	Reproduction Factor	Nematodes per root gram
σ_a^{2a}	13.1238	0.5622	11.2973
σ_a^{2b}	401.5690	24.7453	500.8805
$\sigma^2_{plots^c}$	0.0955	0.0167	0.2839
σ_e^{2d}	141.4035	4.4519	114.3441
σ_f^{2e}	556.1918	29.7760	626.8058
$\hat{h}_a^{2f}$	0.0236	0.0188	0.0180
$\hat{h}_g^{2g}$	0.7456 ± 0.1495	0.8499 ± 0.1596	0.817 ± 0.1565
$\hat{h}_d^{2h}$	0.7220	0.8310	0.7991
$C^2_{plots^i}$	0.002	0.006	0.005
Overall meanpopulation	104. 0917	6.1129	19. 5720
BR 106 mean	74.87	29.40	86.58

*REML = Restricted Maximum Likelihood / Best Linear Unbiased Prediction; [a]Estimates of additive genetic variance; [b]dominance genetic variance; [c]environmental variance between plots; [d]residualvariance; [e]individual phenotypic variance; [f]individual narrow-sense heritability; [g]individual broad-sense heritability; [h]individual heritability of the dominance effects; [i]coefficient of determination of the plot effects.

Estimates of genetic parameters and individual selection using REML / BLUP procedure

Estimates of coefficient of broad sense heritability that captured the total genotypic effects were higher than 74 % for root mass, reproduction factor and nematodes per gram of root, especially for the reproduction factor (84 %) having the highest value (Table 6). The high values for these traits show great potential for genetic progress in the selection of individuals resistant to *P. brachyurus*.

Estimates of genetic variances were primarily derived from dominance effects for root mass (401.57), reproduction factor (24.745) and nematodes per gram of root (500.881). Consequently, the individual heritability of dominance was increased, ranging from 0.722 to 0.831. Such values are close to estimates of broad sense heritabilities (Table 6). The narrow sense individual heritabilities were low for the three traits, ranging from 0.018 to 0.024, as evidenced by low estimates of additive variances, reaching values up to 13.124 for root mass (Table 6).

Considering phenotypic variances of root mass (556.192), reproduction factor (29.776) and nematodes per gram of roots (626.806), dominance variances were higher than residuals, ranging from 4,452 (reproduction factor) to 141,404 (root mass). These values corroborate that these conditions are favorable for selection and demonstrate that most parts of the phenotypes are attributed to genetic causes of dominance effects (Table 6).

Determination coefficients of plot effects ranged from 0.002 to 0.006 for all traits. The low values of these coefficients are attributed to very small environmental variations within the plots and indicate good experimental precision, corroborated by low estimates of environmental variance between the plots, ranging from 0.096 to 0.284 (Table 6).

A significant different was observed for genotypic values of hybrids for the reproduction factor of nematodes, which ranged from 0.63 to 18.48 (Table 7). The following hybrids stand out for the selection of resistant individuals based on their genotypic values (u + g): CH3.2, CH3.23, CH3.8, CH3.37, CH3.38, CH3.41 and CH3.36 from cross 06354-047 × Nocera; CH2.1 and CH2.7, from cross 06354-047 × Cereza; and CH1.1, CH1.3 and CH1.2 from cross 06354-047 × Nocera.

Discussion

Based on the pseudo-F and pseudo-t2 criteria, the likelihood function defined that the ideal number of homogeneous groups is three. These groups were formed according to their similarities, which is mainly due to the greater increase in the likelihood function observed for group III, that is, 65.54 (Figure 1).

The likelihood function was used to define more accurate criteria for the formation of the three groups, which resulted in more objective groupings than the hierarchical grouping methods in which the number of groups is established in a more subjective manner (Gonçalves et al., 2008; Kurosawa et al., 2017).

For most hybrids allocated to each group formed, there was predominance of hybrids with leaves with weak anthocyanin pigmentation in the main veins of the upper limb. However, 35 % of the hybrids had pigmentation varying from medium to strong levels. Similarly, 29 % of these hybrids had anthocyanin pigmentation varying from medium to strong levels in the young branches (Table 3). In red grapes, high concentration of anthocyanins in leaves and branches are important for production of juice and red wine, because anthocyanins contribute to sensory attributes and mainly to color and flavor. However, quantity and composition of these anthocyanins differ according to species, variety, phenological stage and climate (Muñoz-Espada et al., 2004).

The knowledge of morphological traits assessed in this work (Table 2, Figure 2) is fundamental for plant breeding programs. These traits may not play a direct role in selection and development of new wine grapes; however, they do contribute to a better characterization of genetic diversity, exploration of genetic variability and subsequent conservation of species.

Vitis hybrids were separated into three groups according to their genotypic values that reactto *P. brachyurus.* Regarding the trait reproduction factor (RF): 46 genotypes were susceptible ($RF \geq 1$); seven were moderately resistant ($1.0 \geq RF < 1.5$); and four, resistant ($RF \leq 1$). Eleven moderately resistant and resistant hybrids selected corresponded to 19 % of the total segregating populations (Table 7). Resistant hybrids derived from crosses that used 06354-047 as a parent, indicating that it may be the source of resistance to *P. brachyurus*.

Groups I and II had resistant hybrids; however, neither was immune to nematodes. The hybrids selected in our study have a dual application, that is, both for direct use as rootstocks or development of fruiting varieties.

Heritability is one of the most important genetic parameters, as it quantifies the fraction of the inheritable phenotypic variation available for selection. Nematodes per gram of root and root mass allow the best genetic progress of broad sense heritability for the traits of reproduction factor. In addition, since the results found that these traits are correlated, there is a good chance of success in advancing all three characters through selection of the reproduction factor alone (Santos et al., 2017; Santos et al., 2015;Kouassi et al., 2009).The sexual reproduction of these hybrids is not likely to provide significant genetic gains for these traits in grape breeding. However, genetic gains can be maximized (Kouassi et al., 2009; Piepho et al., 2009) by selecting hybrids for lower reproduction factor and nematodes per gram of root as well as by cloning the material selected. The number of nematodes per gram of fresh roots is a good parameter to assess nematode populations, since it correlates directly with losses caused by nematodes (Starr et al., 2002).

Table 7 – Arrangement of 57 interspecific hybrids of *Vitis* spp. for resistance to nematode *P. brachyurus:* where (a) refers to predicted additive effects, (d) predicted dominance effects, (g) predicted genotypic effects, (u+g) genotypic average, genetic gain, and new average or genotypic values for trait reproduction factor in the selection of hybrids. Data acquired and analyzed at UENF, Campos dos Goytacazes, Rio de Janeiro, Brazil, 2016

Order	Hybrids	a	d	g	u + g	Gain	New Average
1	CH1.2	-0.0962	-5.3813	-5.4774	0.6355	0.0063	6.1193
2	CH3.36	-0.0700	-5.3257	-5.3956	0.7173	0.1043	6.2172
3	CH2.7	-0.0631	-5.1453	-5.2083	0.9046	0.2043	6.3172
4	CH3.38	-0.0663	-5.0817	-5.1479	0.965	0.3045	6.4174
5	CH1.3	-0.0905	-5.003	-5.0935	1.0194	0.4074	6.5203
6	CH3.37	-0.0642	-4.9428	-5.0069	1.106	0.5132	6.6261
7	CH1.1	-0.0891	-4.911	-5.0001	1.1129	0.6214	6.7343
8	CH3.8	-0.0633	-4.8855	-4.9488	1.1641	0.7338	6.8468
9	CH2.1	-0.0584	-4.8349	-4.8932	1.2197	0.8498	6.9627
10	CH3.23	-0.0605	-4.7036	-4.7641	1.3488	0.9694	7.0824
11	CH3.2	-0.0597	-4.6493	-4.7091	1.4039	1.0914	7.2044
12	CH3.41	-0.0493	-3.9642	-4.0136	2.0993	1.2175	7.3305
13	CH3.6	-0.0423	-3.499	-3.5413	2.5716	1.3338	7.4467
14	CH3.1	-0.0371	-3.1594	-3.1966	2.9164	1.4446	7.5575
15	CH3.9	-0.0358	-3.0694	-3.1052	3.0077	1.5525	7.6654
16	CH3.5	-0.0333	-2.9085	-2.9418	3.1711	1.6634	7.7763
17	CH3.13	-0.031	-2.7517	-2.7827	3.3302	1.7757	7.8887
18	CH3.16	-0.0259	-2.4205	-2.4464	3.6665	1.8897	8.0026
19	CH3.32	-0.0255	-2.3936	-2.4192	3.6937	2.0009	8.1138
20	CH3.3	-0.0236	-2.2642	-2.2878	3.8252	2.1172	8.2301
21	CH3.30	-0.0215	-2.1252	-2.1467	3.9663	2.2363	8.3492
22	CH2.2	-0.0166	-2.0788	-2.0954	4.0175	2.358	8.4709
23	CH3.24	-0.0119	-1.4905	-1.5024	4.6106	2.4852	8.5982
24	CH3.34	-0.0081	-1.2407	-1.2488	4.8641	2.6025	8.7155
25	CH3.10	-0.0066	-1.1472	-1.1539	4.9591	2.7192	8.8322
26	CH3.27	-0.0041	-0.9821	-0.9863	5.1267	2.8403	8.9532
27	CH3.25	-0.0031	-0.916	-0.9192	5.1938	2.9637	9.0766
28	CH3.12	0.0041	-0.4376	-0.4335	5.6794	3.0931	9.2061
29	CH3.7	0.0046	-0.4028	-0.3981	5.7148	3.2147	9.3277
30	CH3.28	0.0048	-0.3936	-0.3889	5.7241	3.3438	9.4567
31	CH3.35	0.0054	-0.3493	-0.3439	5.7691	3.482	9.5949
32	CH3.20	0.0055	-0.3467	-0.3412	5.7717	3.6292	9.7421
33	CH3.14	0.0143	0.2382	0.2526	6.3655	3.788	9.9009
34	CH2.9	0.0187	0.2505	0.2692	6.3821	3.9353	10.0482
35	CH2.6	0.0202	0.3503	0.3705	6.4834	4.0947	10.2076
36	CH2.5	0.0264	0.7609	0.7873	6.9003	4.264	10.3769
37	CH1.4	-0.0023	0.8154	0.8131	6.9261	4.4295	10.5424
38	CH3.18	0.0243	0.8952	0.9195	7.0324	4.6103	10.7233
39	CH3.31	0.0273	1.0956	1.1229	7.2359	4.8046	10.9175
40	CH3.4	0.0299	1.2661	1.296	7.409	5.0091	11.1221
41	CH3.22	0.0371	1.7385	1.7756	7.8885	5.2275	11.3405
42	CH1.5	0.0128	1.8131	1.8259	7.9388	5.4433	11.5562
43	CH1.3	0.0258	2.6683	2.6941	8.8071	5.6845	11.7974
44	CH3.15	0.0532	2.7998	2.853	8.9659	5.898	12.011
45	CH3.11	0.0542	2.8685	2.9227	9.0356	6.1323	12.2452
46	CH2.4	0.0599	2.9672	3.0271	9.14	6.3997	12.5127
47	CH3.26	0.0601	3.256	3.316	9.429	6.7064	12.8193
48	CH3.17	0.0765	4.3423	4.4188	10.5317	7.0454	13.1583
49	CH3.29	0.0779	4.4319	4.5098	10.6227	7.3372	13.4502
50	CH3.33	0.082	4.702	4.7839	10.8969	7.6907	13.8036
51	CH3.39	0.0863	4.9862	5.0725	11.1854	8.1059	14.2188
52	CH1.7	0.0636	5.1635	5.2271	11.34	8.6115	14.7244
53	CH2.3	0.1144	6.5668	6.6812	12.7941	9.2884	15.4013
54	CH2.8	0.1266	7.3685	7.4951	13.608	9.9401	16.0531
55	CH3.19	0.1489	9.117	9.2659	15.3788	10.755	16.8681
56	CH3.40	0.1692	10.4602	10.6295	16.7424	11.499	17.6127
57	CH3.21	0.1952	12.1749	12.3702	18.4831	12.370	18.4831
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In this work, the conditions are favorable for selection since most phenotypes are attributed to genetic causes. The total phenotypic variances tended towards zero, due to the environmental effect between plots and determination coefficients of plot effects. Thus, it can be concluded that there is a small magnitude effect of plots and good selection efficiency regarding experimental precision for reproduction factor, root mass and nematode per gram of root (Santos et al., 2017).

For selection, using the average components predicted by BLUP, plant breeders should prioritize genotypic values, as these are necessary predicted true values (Piepho et al., 2009). Thus, selected hybrids should have average reproduction factors of up to 1.40 (Table 7).The use of reproduction factor for selection demonstrated that most hybrids are susceptible since they had reproduction factor values higher than 1.0. However, for several hybrids, the genetic values (u + g) for reproduction factor ranged from1.0 to2.0. In these cases, it is advisable to consider susceptibility as moderate resistance, since they are genotypes that allow nematode survival, without showing strong symptoms of their feeding. The same is applied to resistance found in hybrid CH3.38, whose genetic value was slightly less than 1.0 for reproduction factor.

On the other hand, several hybrids, with reproduction factor values above or very close to 2.0 should be considered susceptible. These individuals are efficient hosts and favor the establishment of *P. brachyurus* populations that are high enough to cause significant direct damage to grapevine growth and prevent their use in breeding programs.

Narrow sense heritability for root mass, nematodes per gram of root and reproduction factor were less than 2 % or almost null for these traits. This reveals that for grapevine breeding, selection for resistance to *P. brachyurus* by sexual reproduction in a population composed of these clones does not lead to genetic gains and is therefore impracticable. While in the broad sense, considering the additive and dominant variances, where heritabilities ($\hat{h}_g^{2g}$) reached values ranging from 74 to 84 %, meaning that selection based on these traits and cloning the selected material can be maximized the genetic gains. In other words, broad sense heritability relative to traits of root mass, nematodes per gram of root and reproduction factor had the greatest genetic advances associated to the cloning of selected genotypes for those traits. The indi-

viduals selected for resistance in this study had the lowest genotypic values, with predicted gains below 1 %. Hybrid CH1.2 had the lowest genotypic value for reproduction factor (u + g = 0.6355) and genetic gain close to zero (0.0063). This indicates that genotypes with lower values for reproduction factor can be cloned and advanced.

The use of resistant grapevines, along with other measures of nematode control, decreases *P. brachyurus* populations in the soil and increases commercial yield of vineyards. Resistant clones can now be evaluated for use as fruiting varieties or as commercial rootstocks (Walker etal., 1994).

Conclusions

The Ward-MLM classification strategy effectively discriminated among the variable genotypes and allowed the separation of 57 genotypes into three homogeneous groups. Groups II and III were the closest genetically and contained hybrids resistant to *P. brachyurus*. High estimates of dominance variance suggest that further genetic progress can be achieved through the cloning of resistant hybrids, more than their use as resistant parents. Based on the genotypic values for reproduction factor, the following resistant and moderately resistant clones were selected: CH3.2, CH3.23, CH3.8, CH3.37, CH3.38, CH3.41, CH3.36, CH2.1, CH2.7, CH1.1, CH1.3 and CH1.2. The hybrids with the lowest nematode populations per gram of root and lower breeding capacity for *P. brachyurus* resistance should be tested as rootstocks and evaluated for use in infested areas.

Acknowledgements

We are thankful to FAPERJ – Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro for the granting and CAPES, of the doctoral scholarship and financial support provided to the first author, as well as to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the accomplishment of the present work.

Authors' Contributions

Conceptualization: Santos, P.R.; Viana, A.P.; Gomes, V.M.; Walker M.A. Data acquisition: Santos, P.R.; Preisigke, S.C.; Almeida, O.F.; Rodrigues, D.L.; Data analysis: Santos, P.R.; Viana, A.P.; Design of Methodology: Santos, P.R.; Viana, A. P.; Gomes, V.M.; Rodrigues, R.; Software development: Santos, P.R.; Viana, A.P.; Writing and editing: Santos, P.R.; Viana, A.P.; Santos, E.A.; Rodrigues, R.; Walker M. A.

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