

Clonal selection in interspecific *Vitis* spp. hybrids resistant to the root-lesion nematode *Pratylenchus brachyurus* by REML/BLUP

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Summary

Introduction – The disorders caused by nematodes reduce in 20 to 25% of the annual production of grapes causing great loss to the crop. In order to select grapes for genetic resistance to *Pratylenchus brachyurus*, we estimated the genetic and environmental coefficients of variation, the individual broad-sense heritability, and the heritability of means of clones; and predicted the genetic values ($u+g$) of interspecific hybrids resistant to that nematode. **Materials and methods** – Three segregating populations from interspecific crosses were evaluated, including 57 hybrids from the crosses between the following parents originating from the Germplasm Bank of the University of California: [C166-043 (*Vitis romanetii*) × 07355-075 (*V. vinifera*)], [06354-047 (*V. vinifera*/*V. rotundifolia*) × Cereza (*V. vinifera*)], and [06354-047 (*V. vinifera*/*V. rotundifolia*) × Nocera (*V. vinifera*)]. The experiment was evaluated as a randomized-block design with two replicates and three plants per plot. Plants were inoculated with suspension of 600 *P. brachyurus* species. The genetic parameters and genetic values were determined using the Restricted Maximum Likelihood/Best Linear Unbiased Prediction (REML/BLUP) procedure for the traits 'root mass', 'nematodes per gram of roots', 'reproduction factor', and 'reproduction index'. **Results and discussion** – The ratio between the genetic and environmental coefficients of variation and the individual broad-sense heritability and the heritability of means of clones were higher than 74% for all traits, indicating efficiency and optimization in the employed procedure for the selection conditions. **Conclusion** – The lower genotypic values ($u+g$) of the 'reproduction factor' and 'reproduction index' traits predicted by BLUP were used to select 14 interspecific hybrids resistant to *P. brachyurus*.

Keywords

Brazil, grape, *Vitis* spp., genetic × environment interaction, genotypic values, pest resistance, vine

Résumé

Sélection clonale par REML/BLUP sur les hybrides interspécifique de *Vitis* spp.

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Significance of this study

What is already known on this subject?

- Nematodes seriously affect grapevines, hindering their development, decreasing their vigor and greatly reducing their root system. The inclusion of wild species in breeding programs is one of the key approaches to pest and disease resistance breeding.

What are the new findings?

- Further genetic progress can be achieved through the cloning of resistant hybrids, more so than their use as resistant parents. Based on the genotypic values for the reproduction factor and reproduction index, resistant and moderately resistant clones were selected.

What is the expected impact on horticulture?

- The use of resistant grapevines, together with other measures of nematode control, will decrease *Pratylenchus brachyurus* populations in the soil and increase the commercial yield of vineyards. Resistant clones can now be evaluated for use as fruiting varieties or as commercial rootstocks.

résistants au nématode des racines
Pratylenchus brachyurus.

Introduction – Les désordres causés par les nématodes réduisent de 20 à 25% la production annuelle de raisins causant de lourdes pertes au vignoble. Dans le but de sélectionner la résistance génétique de la vigne à *Pratylenchus brachyurus*, nous avons estimé les coefficients de variation génétique et environnemental, l'héritabilité au sens large et l'héritabilité moyenne des clones, et prédit les valeurs génétiques ($u+g$) des hybrides interspécifiques résistants à ce nématode. **Matériel et méthodes** – Trois populations ségrégeant à partir de croisements interspécifiques ont été évaluées, dont 57 hybrides provenant des croisements entre les parents suivants provenant de la banque de gènes de l'Université de Californie: [C166-043 (*Vitis romanetii*) × 07355-075 (*V. vinifera*)], [06354-047 (*V. vinifera* / *V. rotundifolia*) × Cereza (*V. vinifera*)], et [06354-047 (*V. vinifera* / *V. rotundifolia*) × Nocera (*V. vinifera*)]. L'expérience a été éva-

luée selon un dispositif en blocs randomisés à deux répétitions et trois plantes par parcelle. Les plantes ont été inoculées avec une suspension de 600 espèces de *P. brachyurus*. Les paramètres génétiques et les valeurs génétiques ont été déterminés en utilisant la procédure de prédiction maximale restreinte / meilleure prédiction linéaire non biaisée (REML/BLUP) pour la masse racinaire des caractères, le nombre de nématodes par gramme de racines, le facteur de reproduction et l'indice de reproduction. *Résultats et discussion* – Les rapports entre les coefficients de variation génétique et environnementale et l'héritabilité au sens large et l'héritabilité des moyennes des clones étaient supérieurs à 74% pour tous les caractères, indiquant l'efficacité et l'optimisation de la procédure utilisée pour les conditions de sélection. *Conclusion* – Les valeurs génotypiques ($u+g$) inférieures des caractères 'facteur de reproduction' et 'indice de reproduction' prédits par BLUP ont été utilisées pour sélectionner 14 hybrides interspécifiques résistants à *P. brachyurus*.

Mots-clés

Brésil, vigne, *Vitis* spp., interaction génétique × environnement, valeurs génotypiques, résistance aux ravageurs

Introduction

The Brazilian viticulture has expanded over the years, especially in new production regions, which include coastal areas, with extremely tropical climate (Camargo *et al.*, 2011; IBGE, 2015). However, to increase or even ensure the productivity and quality of fruits, it is necessary to develop new cultivars adapted and resistant to the main diseases in areas of introduced and traditional crops.

Disorders caused by phytonematodes greatly affect the vine, especially under high populations of the pathogen in the soil. Amidst the phytonematodes, the root lesion nematode (*Pratylenchus* spp.) is considered a determining factor to the low worldwide and Brazilian production (Naves *et al.*, 2005; McKenry and Anwar, 2006; Ferris *et al.*, 2012). To this date, 70 *Pratylenchus* species have been discovered. In Brazil, the following species have been recorded: *P. coffeae*, *P. brachyurus*, *P. zaeae*, *P. penetrans*, *P. neglectus*, *P. scribneri*, *P. vulnus*, *P. pseudopratensis*, *P. jordanensis*, *P. pseudofallax*, and *P. jaehni*. Of this genus, three species cause lesions to the vine roots: *P. brachyurus*, *P. jordanensis*, and *P. thornei*; within this group, *P. brachyurus* is noteworthy for its broad dissemination across the main agricultural crop areas and is currently recognized as one of the main problems in viticulture (Naves, 2005; Ferris *et al.*, 2012; Puerari *et al.*, 2012; Zasada *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 the control of the pathogen in the vine, as it does not elevate production costs and nor does it harm the environment (Pinheiro *et al.*, 2009). For this reason, there is a demand for the selection of resistant clones for the generation of high yields, since the genetic breeding aimed at incorporating resistance to *P. brachyurus* encounters difficulties because the species is polyphagous, little specialized, and of migratory endoparasitic habit, which causes it to not fix itself onto the host plant (Inomoto *et al.*, 2007;

Goulart, 2008; Puerari *et al.*, 2012). However, interspecific hybridization in breeding programs is a strategy to address these problems when wild species are included; these species may contain the genes that provide genetic resistance to *P. brachyurus* not found in cultivated vines (Schuck *et al.*, 2011; Santos *et al.*, 2015).

In this sense, genetic variability for the selection of resistant genotypes in interspecific segregating populations is particularly important to the breeder, since the magnitude of genetic variability available in these populations is quantified and exploited in breeding programs (Schuck *et al.*, 2011; Santos *et al.*, 2015). Estimates of genetic parameters such as heritabilities and genotypic and environmental coefficients of variation are essential for knowing the genetic nature involved in the control of traits and enable the selection of genotypes (Resende *et al.*, 2016; Santos *et al.*, 2017). In unbalanced experiments – a common situation in experimentation in the field with fruit trees – analysis of variance leads to inaccurate estimates of variance components, which makes the estimates of genetic values unreliable. In these cases, the Restricted Maximum Likelihood/Best Linear Unbiased Prediction (REML/BLUP) procedure is an optimized method of genotypic assessment (Viana and Resende, 2014). In vines, statistical methods adopting this approach for the estimate of genetic parameters and components of genotypic means ($u+g$) for the 'reproduction factor' and 'reproduction index' traits, which define genetic resistance to nematodes, are not exploited. However, the present study fills this gap through this pioneering approach in vine breeding.

In this study, we estimated the genetic and environmental coefficients of variation, the broad-sense individual heritability, and the heritability of means of clones; and predicted the genetic values ($u+g$) using the REML/BLUP procedure for selection efficiency of interspecific hybrids resistant to the nematode *P. brachyurus*.

Materials and methods

Interspecific hybrids evaluated

Three segregating populations of interspecific crosses of *Vitis* spp. were evaluated for resistance to *P. brachyurus*. In total, 57 hybrids originating from the cross between the following parents were evaluated:

- [C166-043 (*V. rotundifolia*) × 07355-075 (*V. vinifera*)] – 7 hybrids;
- [06354-047 (*V. vinifera*/*V. rotundifolia*) × Cereza (*V. vinifera*)] – 9 hybrids; and
- [06354-047 (*V. vinifera*/*V. rotundifolia*) × Nocera (*V. vinifera*)] – 41 hybrids.

The obtained crosses and hybrids originate from the Active Germplasm Bank of the Department of Viticulture and Enology at the University of California.

Establishment of the experiment, inoculation, and evaluation

The obtained hybrid cuttings were rooted into pots with 7-L capacity containing a soil:sand mixture at the ratio of 2:1 (v:v) and kept for establishment of the seedlings in a greenhouse at the Unit for Research Support at the State University of Northern Rio de Janeiro. A randomized-block design was adopted with two replicates and three plants per plot (of the 57 interspecific *Vitis* hybrids plus corn cultivar 'BR 106' [susceptibility standard], which was used as control to check the viability of the inoculant source and for the estimates of the nematode reproduction index in the vine hybrids).

After four months (November 2015), the plants were inoculated with suspension of 600 specimens of *P. brachyurus*. The nematode suspension was calibrated to 300 nematodes mL⁻¹ and distributed into two orifices open around the grape and corn seedlings. The corn was evaluated at approximately 90 days after inoculation, and each pot received a new plant, which was evaluated together with the grape plants. At 180 days after inoculation, a destructive method was used, and the root system of the grape and corn plant was collected for nematode extraction. In the roots, nematodes were extracted by using the methodology proposed by Coolen and D’Herde (1972). The obtained samples were evaluated by counting all *P. brachyurus* specimens present in each sample under a stereoscopic microscope, using a Peters’ slide.

Evaluated traits

The evaluated traits were root mass, nematodes per gram of root, reproduction factor, and reproduction index. The reproduction factor was estimated as $RF = F_p / I_p$, where RF , F_p , and I_p correspond to the reproduction factor and the final and initial populations, respectively. Plants were classified as immune ($RF = 0$), resistant ($0 < RF < 1$), and susceptible ($RF > 1$), following Oostenbrink’s (1966) criterion. The nematode distribution index was determined based on the reproduction of nematodes in the corn as control (100%) in comparison with the vine hybrids, according to the methodology established by Taylor (1967). The final population (F_p) found in the grape genotypes was divided by that found in corn, to define the reproduction index (RI). Based on these values, we defined the level of resistance of each grape genotype to *P. brachyurus*, according to the following reproduction criterion defined by Taylor (1967): S - susceptible plant, normal reproduction, RI above 51%; SR - slightly resistant, RI from 26 to 50%; MR - moderately resistant, RI between 11 and 35%; VR - very resistant, RI from 1 to 10%; and ER/I - extremely resistant/immune, RI below 1%.

Statistical analysis of the traits

To choose the representative value of a clone, the genotypic values for each evaluated trait were obtained based on the REML procedure applied to the following mixed linear model, used in matrix form for the assessment of related clones in the randomized-block design with several plants per plot, suggested by Viana and Resende (2014):

$$Y = Xr + Za + Zd + Wp + e$$

where Y is the vector of data; r is the vector of the replicate effects (assumed as fixed) added to the overall mean; a is the vector of additive genetic effects (assumed as random); d is the vector of dominance effects (assumed as random); p is the vector of plot effects; and e is the vector of errors or residuals (random). Uppercase letters represent the incidence matrices for the said effects.

The significance of random effects was obtained by an analysis of deviance (ANADEV) via the REML method; the significance of the effects of the model was estimated by deviance analysis, as recommended by Viana and Resende (2014), which replaces the analysis of variance (ANOVA) with advantages in cases of unbalanced data, by the maximum Likelihood Ratio Test (LRT). The deviances were obtained using the model with and without the effects clones and plots, subtracting from each deviance obtained in the full model from the model without this effect and comparing it with the Chi-square (χ^2) value with one degree of freedom, at

1% and 5% probability. Mathematically:

$$LRT = -2 \ln \left(\frac{ML \text{ of the residual model}}{ML \text{ of the completed model}} \right)$$

where \ln is logarithm neperian and ML is the maximum likelihood. The block effect, assumed fixed, was evaluated by Snedecor’s F test (Viana and Resende, 2014; Silva *et al.*, 2017).

The following genetic parameters were studied: genetic variance (σ_g^2), environmental variances (σ_e^2), experimental coefficient of variation $CVe\%$, genetic coefficient of variation ($CVg\%$), variation index ($IV = CVg/CVe$), individual broad-sense heritability (\hat{h}_g^2), heritability of mean of clones (\hat{h}_m^2), and overall mean of the experiment for each trait.

The presented system predicts the additive (\hat{a}) and dominance (\hat{d}) effects separately. The total genotypic values, given as $\hat{g} = \hat{a} + \hat{d}$, can be predicted directly by mixed-model equations:

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

where $G = \sigma_a^2 + D\sigma_a^2$. The genetic (CVg) and environmental (CVe) coefficients of variation as well as the variation index (CVg/CVe) for the evaluated traits were estimated based on the following expressions, according to the procedure described by Vencovsky and Barriga (1992):

$$CVg\% = \frac{\sqrt{\sigma_g^2}}{\mu} \times 100; CVe\% = \frac{\sqrt{\sigma_e^2}}{\mu} \times 100; \text{ and } VI = \frac{CVg}{CVe}$$

After the means corrected by the BLUP procedure were obtained, the genetic correlations among the analyzed variables were estimated.

Results and discussion

Variability of resistance among the hybrids

The significance of genotypic effects measured here was evaluated by deviance analysis, which showed significance for the effects of hybrids at the 1% probability level for all traits (Table 1). This reveals the existence of variability among the evaluated interspecific hybrids; in other words, their effects explain part of the total variation, which demonstrates the possibility of obtaining genetic gains through selection for resistant genotypes. For all traits, the plot effects showed values close to zero, *i.e.*, not significant at the 5% significance level, indicating that a small environmental variation remained within the plots and that selection should take into account the means of clones.

The environmental coefficients of variation were high for reproduction factor, nematodes per gram of roots, and reproduction index: 34.47, 42.86 and 34.76%, respectively (Table 2). However, coefficients of such magnitudes are common in experiments with nematodes (Cardoso *et al.*, 2005; Aballay *et al.*, 2009), whereas for root mass, the environmental coefficient of variation was medium (11.16%), demonstrating good experimental precision for this trait (Pimentel-Gomes, 2009). Higher estimates for the genetic coefficient of variation were observed for root mass, reproduction factor, nematodes per gram of roots, and reproduction index, which indicates, besides high genetic variability among the interspecific hybrids, a situation very favorable to the selection of clones resistant to *Pratylenchus brachyurus*.



TABLE 1. Deviance analysis of the traits: root mass (g), reproduction factor, nematodes per gram of root and reproduction index from 57 interspecific hybrids of *Vitis* spp. for resistance to the nematode *P. brachyurus*.

Effects	Root mass		Reproduction factor	
	Deviance	LRT (χ^2) ²	Deviance	LRT (χ^2)
Clones ¹	1,473.48	713.41**	806.94	1,231.42**
Plot ¹	2,186.85	0.04 ^{ns}	2,036.04	2.32 ^{ns}
Full model	2,186.89	-	2,038.36	-
Effects	Nematodes g ⁻¹ roots		Reproduction index	
	Deviance	LRT (χ^2)	Deviance	LRT (χ^2)
Clones ¹	1,467.9	1,007.55**	1,710.37	1,231.57**
Plot ¹	2,474.35	1.10 ^{ns}	2,939.61	2.33 ^{ns}
Full model	2,475.45	-	2,941.94	-

¹ Deviance of the adjusted model, without those effects.

² LRT: Likelihood ratio test, distribution with 1 degree of freedom.

^{ns}: Not significant; **: Significant at 1% probability, by the test of (χ^2) (1% = 6.63; 5% = 3.84).

TABLE 2. Genetic parameters of the traits: root mass (g), reproduction factor, nematodes per gram of root and reproduction index, from 57 interspecific hybrids of *Vitis* spp. for resistance to the nematode *P. brachyurus*.

Genetic parameters ^y	Traits			
	Root mass	Reproduction factor	Nematodes g ⁻¹ roots	Reproduction index
σ_g^2	414.69	25.31	512.18	1,948.50
σ_e^2	141.40	4.45	114.34	343.38
CVe %	11.16	34.47	42.86	34.76
CVg %	19.11	82.20	90.70	82.32
IV = CVg/CVe	1.71	2.38	2.12	2.37
\hat{h}_g^2 (%)	74.56	84.99	81.71	85.01
\hat{h}_m^2 (%)	85.44	92.73	89.96	91.90
General average ^z	106.54	6.12	24.95	53.62

^y Estimates of genetic variances (σ_g^2); Environmental variance (σ_e^2); Experimental coefficient of variation (CVe%); Genetic coefficient of variation (CVg%); Variation index (IV = CVg/CVe); Individual broad-sense heritability (\hat{h}_g^2); Heritability of mean of clones (\hat{h}_m^2).

^z Overall mean of the experiment.

Heritability of nematode resistance

The estimates of individual broad-sense heritability, which captures the total genotypic effects, were high for all traits, ranging from 74.56 to 85.01%. They reflect the fact that most parts of the phenotypic variability observed had a genetic nature, and indicate that selection based on these traits could be efficiently performed; this means low reproduction as well as smaller nematode populations in the vine's rhizosphere and larger root mass. This fact is reinforced by the estimated variation indices, which were all greater than 1.0, which also indicates a situation favorable for the selection of resistant clones, especially for the traits 'reproduction factor', 'nematodes per gram of roots', and 'reproduction index', whose proportion of genetic variation was 2.12 to 2.38 times larger than that of environmental variation (Vencovsky and Barriga, 1992; Santos *et al.*, 2017).

For all traits, we obviously obtain $\hat{h}_m^2 > \hat{h}_g^2$, *i.e.*, selection based on means of clones is at a higher level of precision than selection based on plots or individual plants. This is due mainly to the influence of experimental errors when we use means rather than individuals as a selection criterion (Vencovsky and Barriga, 1992). Based on the obtained results, in fact, only \hat{h}_m^2 was interesting to evaluate the selection expect-

tations, since no individual clones were selected based on the samplings within plots, coupled with the fact that in this type of population, genetic variance is expected to be obtained only among the tested clones, since the propagation method already fixes the traits of the individuals that are cloned. The \hat{h}_g^2 coefficient is only valid for purposes of comparison with others, because it is independent of number of replicates and number of individuals within plots.

Reproduction factor as key indicator

A large difference was observed among the evaluated hybrids in terms of genotypic values ($u+g$), which ranged from 0.63 to 18.48 for the nematodes' reproduction factor (Table 3). For a selection effect by the components of the mean predicted by BLUP, researchers must choose genotypic values ($u+g$) over phenotypic means, in plant breeding, since they are the true values to be predicted (Borges *et al.*, 2009; Viana and Resende, 2014).

According to the criterion mentioned by Oostenbrink (1966), based on the genotypic values, 92.98% of the tested interspecific hybrids were considered susceptible, with a reproduction factor equal to or greater than 1.0 (Table 3). The hybrids from the cross [06354-047 × Cereza], C2.3

TABLE 3. Genotypic values ($u+g$) for the traits reproduction factor and reproduction index, of the 57 interspecific hybrids of *Vitis* spp. for resistance to the nematode *P. brachyurus*.

Clones	Crossings	Reproduction factor		Reproduction index	
		$u+g$	Category ^a	$u+g$	Category ^b
C1.1	[<i>Vitis</i>	1.12	S	9.75	VR
C1.2	<i>romanetii</i>	0.64	R	5.57	VR
C1.3	C166-043 ×	8.81	S	77.27	S
C1.4	07355-075]	6.93	S	60.72	S
C1.5		7.94	S	69.66	S
C1.6		1.02	S	8.94	VR
C1.7		11.34	S	99.49	S
C2.1	[06354-047	1.22	S	10.68	VR
C2.2	× Cereza]	4.02	S	35.22	SR
C2.3		12.79	S	112.24	S
C2.4		9.14	S	80.2	S
C2.5		6.90	S	60.54	S
C2.6		6.48	S	56.89	S
C2.7		0.90	R	7.92	VR
C2.8		13.61	S	119.41	S
C2.9		6.38	S	55.99	S
C3.1	[06354-047	2.92	S	25.57	MR
C3.2	× Nocera]	1.40	S	12.3	MR
C3.3		3.83	S	33.55	SR
C3.4		7.41	S	65.01	S
C3.5		3.17	S	27.8	SR
C3.6		2.57	S	22.56	MR
C3.7		5.71	S	50.14	SR
C3.8		1.16	S	10.2	VR
C3.9		3.01	S	26.38	SR
C3.10		4.96	S	43.48	SR
C3.11		9.04	S	79.28	S
C3.12		5.68	S	49.81	SR
C3.13		3.33	S	29.21	SR
C3.14		6.37	S	55.84	S
C3.15		8.97	S	78.66	S
C3.16		3.67	S	32.15	SR
C3.17		10.53	S	92.36	S
C3.18		7.03	S	61.7	S
C3.19		15.38	S	134.97	S
C3.20		5.77	S	50.64	SR
C3.21		18.48	S	162.2	S
C3.22		7.89	S	69.22	S
C3.23		1.35	S	11.81	MR
C3.24		4.61	S	40.45	SR
C3.25		5.19	S	45.56	SR
C3.26		9.43	S	82.73	S
C3.27		5.13	S	44.97	SR
C3.28		5.72	S	50.22	SR
C3.29		10.62	S	93.21	S
C3.30		3.97	S	34.79	SR
C3.31		7.24	S	63.48	S
C3.32		3.69	S	32.41	SR
C3.33		10.90	S	95.61	S
C3.34		4.86	S	42.66	SR
C3.35		5.77	S	50.62	SR
C3.36		0.72	R	6.28	VR
C3.37		1.11	S	9.71	VR
C3.38		0.97	R	8.44	VR
C3.39		11.19	S	98.15	S
C3.40		16.74	S	146.92	S
C3.41		2.10	S	18.4	MR
BR 106 (Maize)		11.40	S	-	-

^aS – susceptible plant and R – resistant plant.

^bS – susceptible plant, normal reproduction, reproduction index: $RI > 51\%$; SR – slightly resistant, $26 < RI < 50\%$; MR – moderately resistant, $11 < RI < 35\%$; VR – very resistant, $1 < RI < 10\%$; and ER/I – extremely resistant/immune, $RI < 1\%$.

($u+g = 12.79$) and C2.8 ($u+g = 13.61$), along with hybrids C3.19 ($u+g = 15.38$), C3.21 ($u+g = 18.48$), and C3.40 ($u+g = 16.74$) from the cross [06354-047 × Nocera], displayed the highest genotypic values for the reproduction factor, higher than the mean shown by susceptible control BR 106 (corn). In these genotypes, the nematode population reached a growth of over 12 times relative to the initial population of 600 specimens of the inoculant with *P. brachyurus*.

By the same criterion of Oostenbrink (1966), 8% of the tested genotypes were classified as resistant, with a reproduction factor below 1.0. This group included the interspecific hybrids C1.2 from the cross between parents [*Vitis romanetii* C166-043 × 07355-075] ($u+g = 0.64$), C2.7 from [06354-047 × Cereza] ($u+g = 0.90$), C3.36 and C3.38 from [06354-047 × Nocera] ($u+g = 0.72$ and 0.97 , respectively). This low frequency of hybrids resistant to *P. brachyurus* is due to the low number of hybrids that were obtained from the crosses and evaluated.

Considering only the trait ‘reproduction factor’, 53 genotypes are considered susceptible (reproduction factor $RF \geq 1$) and only four are resistant ($RF \leq 1$). Seven hybrids showed $2 \geq RF \geq 1$, and these can also be selected to increase the truncation point, reaching up to 19.29% in the total selected segregating genotypes. This is because the interspecific hybrids selected in this study are still under testing and show resistance for other diseases like mildew (*Plasmopara viticola*) and powdery mildew (*Uncinula necator*), possibly having double aptitude. In other words, they can be used directly as rootstock and also as crown based on the clones selected and propagated vegetatively.

The reproduction index

According to the reproduction criterion established by Taylor (1967), we obtained from susceptible to very resistant hybrids depending on their reproduction index, when the standard control was used for the nematode species under study (Table 2). Among the tested hybrids, 43.86% were classified as susceptible, including corn cultivar BR106; 31.58% were classified as slightly resistant; 8.77% as moderately resistant; 15.79% as very resistant; and none was extremely resistant or immune. Resistant genotypes of sweet potato (Marchese *et al.*, 2010), common bean, and green bean (Ferreira *et al.*, 2010) were successfully obtained when susceptible controls were used as the standard host, following the same criterion used in the present study, which reveals effectiveness for the classification of genotypes as to genetic resistance to nematodes.

The genotypes classified by the reproduction index as very resistant and moderately resistant accounted for 24.56% of the total, and this includes the same four genotypes classified as resistant based on the reproduction factor ≤ 1 . In this scenario, 14 interspecific hybrids were selected as resistant and will be cloned to provide continuity to the vine breeding program.

The comparison between the criteria of Oostenbrink (1966) and Taylor (1967) reveals consistency, and both are efficient for the identification and selection of genotypes resistant to *P. brachyurus*. However, because the reproduction index allows for a broader distribution of classes – S, SR, MR, HR, and ER/I – greater flexibility is possible for establishing a truncation point of the genotypes to be selected as resistant as compared with the reproduction factor alone. Plant breeding aimed at incorporating resistance to *P. brachyurus* to generate resistant cultivars is considered a difficult goal, because this species is polyphagous, of mi-

TABLE 4. Genotypic values ($u+g$) for the traits root mass (g) and nematodes per gram of root, of the 57 interspecific hybrids of *Vitis* spp. for resistance to the nematode *P. brachyurus*.

Clones	Crossings	Root mass $u+g$	Nematodes g^{-1} roots $u+g$
C1.1*	[<i>Vitis rotundifolia</i> × <i>Vitis vulpina</i>]	117.58	4.5
C1.2*	C166-043 ×	111.93	2.53
C1.3	07355-075]	105.30	30.6
C1.4		100.03	28.49
C1.5		87.53	40.84
C1.6*		74.31	4.85
C1.7		97.43	46.19
C2.1	[06354-047 ×	104.25	4.73
C2.2*	Cereza]	93.77	17.74
C2.3		125.44	37.95
C2.4		174.34	24.89
C2.5		117.42	23.44
C2.6		99.87	26.09
C2.7*		129.47	3.32
C2.8		93.00	59.05
C2.9		89.69	29.39
C3.1*	[06354-047 ×	121.32	9.91
C3.2*	Nocera]	111.96	5.46
C3.3		122.12	12.61
C3.4		90.61	32.97
C3.5		101.56	12.99
C3.6*		130.39	8.10
C3.7		101.53	23.51
C3.8*		86.11	5.81
C3.9		106.4	11.32
C3.10		86.82	23.89
C3.11		98.78	36.87
C3.12		97.83	23.45
C3.13		123.02	11.37
C3.14		110.87	23.52
C3.15		114.12	31.47
C3.16		124.9	12.01
C3.17		109.69	40.49
C3.18		119.29	23.95
C3.19		101.99	60.16
C3.20		105.23	21.94
C3.21		91.87	80.32
C3.22		114.21	27.98
C3.23*		131.14	4.49
C3.24		95.56	20.24
C3.25		103.5	20.29
C3.26		85.70	45.9
C3.27		107.15	19.25
C3.28		103.09	22.45
C3.29		105.00	40.38
C3.30		85.37	18.89
C3.31		129.2	22.32
C3.32		109.57	13.58
C3.33		85.42	51.88
C3.34		91.00	21.83
C3.35		122.55	19.15
C3.36*		89.41	3.39
C3.37*		100.54	5.04
C3.38*		117.68	3.87
C3.39		115.33	39.68
C3.40		77.68	93.94
C3.41*		125.78	7.05
BR 106	(Maize)	85.00	

* Interspecific hybrids selected by genotypic values ($u+g$) based on resistance criteria defined by the Oostenbrink's (1966) reproduction factor and reproduction index by Taylor's (1967) criteria, defined as moderately resistant.

gratory endoparasitic habit, and the wide array of hosts suggests that the parasitism of *P. brachyurus* is little specialized in comparison with other phytonematodes, e.g., *Heterodera* spp. and *Meloidogyne* spp., among others (Starr et al., 2002; Castillo and Vovlas, 2007; Goulart, 2008).

Interaction studies

Overall, the hybrids selected in this study by reproduction factor and reproduction index displayed higher and lower genotypic values ($u+g$) for root mass and number of nematodes g^{-1} roots (Table 4). A similar response was found with the susceptible genotypes, which is explained by the fact that these traits had negative correlations of phenotypic, genetic, and environmental nature (Table 5). However, there is a slight trend for resistant genotypes to have larger root mass and smaller populations of nematodes in the rhizosphere. This happens because despite being of low magnitude, the correlations between root mass and reproduction factor, nematodes g^{-1} , and reproduction index were negative. We also observed that the phenotypic correlations are slightly higher than the genetic correlations because of the higher correlations between the environmental effects of root mass with the other traits. On the other hand, for the environmental effects that affect the reproduction factor × reproduction index, reproduction factor × nematodes g^{-1} roots, and nematodes g^{-1} roots × reproduction index associations, correlations are low, which is why the phenotypic correlations approach the genetic correlations. Results indicate that when it comes to selection of genotypes for resistance, there are great chances of success in the three traits via selection of only the reproduction index, since, according to Falconer and Mackay (1996), when the breeder selects genotypes for traits that contribute positively to the trait of interest, correlation is used more effectively.

Conclusion

The analysis by the REML/BLUP method and the presented model were adequate for the selection of vine genotypes with genetic resistance to *P. brachyurus*. Therefore, the interspecific hybrids resistant to *P. brachyurus* selected in this study could be used as rootstock or cloned to provide continuity to the vine breeding program.

TABLE 5. Phenotypic (r_f), genetic (r_g) and environmental (r_e) correlations between root mass, nematodes per gram of root, reproductive factor and reproduction index of the 57 interspecific hybrids of *Vitis* spp. for resistance to the nematode *P. brachyurus*.

Traits	Correlation coefficients	Reproductive factor	Nematodes g^{-1} root	Reproduction index
Root mass	r_f	-0.1669	-0.3515	-0.1669
	r_g	-0.0598	-0.2655	-0.0597
	r_e	-0.1885	-0.3622	-0.1808
Reproductive factor	r_f		0.9523	0.9985
	r_g		0.9574	0.9985
	r_e		0.6528	0.5985
Nematodes g^{-1} root	r_f			0.9523
	r_g			0.9574
	r_e			0.6520

References

- Aballay, E., Persson, P., and Martensson, A. (2009). Plant-parasitic nematodes in Chilean vineyards. *Nematropica* 31, 85–97.
- Borges, V., Ferreira, P.V., Soares, L., Santos, G.M., and Santos, A.M.M. (2010). Seleção de clones de batatadoce pelo procedimento REML/BLUP. *Acta Sci. Agron.* 32, 643–649. <https://doi.org/10.4025/actasciagron.v32i4.4837>.
- Camargo, U.A., Tonietto, J., and Hoffmann, A. (2011). Progressos na viticultura Brasileira. *Rev. Brasil. de Fruticult. (Volume Especial)*, 144–149. <https://doi.org/10.1590/S010029452011000500017>.
- Castillo, P., and Vovlas, N. (2007). *Pratylenchus* (Nematoda: Pratylenchidae): Diagnosis, biology, pathogenicity and management. *Nematol. Monogr. and Perspect.* 6, 529. <https://doi.org/10.1111/j.1365-3059.2009.02097.x>.
- Coolen, W.A., and D'Herde, C.J. (1972). A Method for the Quantitative Extraction of Nematodes from Plant Tissue. (Ghent, Belgium: Belgium State Agricultural Research Centre), 77 pp.
- Falconer, D.S., and Mackay, T.F.C. (1996). Introduction to Quantitative Genetics (London: Longman Malaysia), 463 pp.
- Ferreira, S., Gomes, L.A.A., Maluf, W.R., Campos, V.P., Carvalho Filho, J.L.S., and Santos, D.C. (2010). Resistance of dry bean and snap bean cultivars to root-knot nematodes. *HortScience* 45, 320–322.
- Ferris, H., Zheng, L., and Walker, M.A. (2012). Resistance of grape rootstocks to plant-parasitic nematodes. *J. Nematol.* 44, 377–386.
- Goulart, A.M.C. (2008). Aspectos gerais sobre nematóides-das-lésões-radiculares (gênero *Pratylenchus*). Documentos 219, July (Planaltina: Embrapa Cerrados).
- IBGE. Instituto Brasileiro de Geografia e Estatística (2015). Banco de dados agregados: produção agrícola municipal, Rio de Janeiro. <http://www.sidra.ibge.gov.br/bda/tabela/listabl.asp?c=106&z=p&o=28> (accessed Dec. 18, 2016).
- Inomoto, M.M., Machado, A.C.Z., and Antedomênico, S.R. (2007). Host status of *Brachiaria* spp. and *Panicum maximum* to *Pratylenchus brachyurus*. *Fitopatol. Brasil.* 32, 341–344. <https://doi.org/10.1590/S0100-41582007000400009>.
- Marchese, A., Maluf, W.R., Gonçalves Neto, A.C., Gonçalves, R.J.S., and Gomes, L.A.A. (2010). Seleção de clones de batata-doce resistentes a *Meloidogyne incognita* raça 1. *Pesq. Agropec. Brasil.* 45, 997–1004. <https://doi.org/10.1590/S0100204X2010000900009>.
- McKenry, M.V., and Anwar, S.A. (2006). Nematode and grape rootstock interactions including an improved understanding of tolerance. *J. Nematol.* 38, 312–318.
- Naves, R.L. (2005). Diagnóstico e manejo de doenças causadas por fitonematóides na cultura da videira (Embrapa Uva e Vinho, Bento Gonçalves).
- Oostenbrink, M. (1966). Major characteristics of the relation between nematodes and plants. *Mededelingen Landbouwhogeschool* 66, 1–46.
- Pimentel-Gomes, F. (2009). Curso de Estatística Experimental, 15th ed. (FEALQ), 451 pp.
- Pinheiro, J.B., Boiteux, L.S., Lopes, C.A., and Silva, G.O. (2009). Identificação de fontes de resistência ao nematoide *Meloidogyne mayaguensis* em acessos de tomateiro (*Solanum lycopersicon*). *Embrapa Hortaliças, Boletim de Pesquisa e Desenvolvimento* 56, 1–19.
- Puerari, H.H., Dias-Arieira, C.R., Moura, M.F., Biela, F., Chiamolera, F.M., and Cunha, T.P.L. (2012). Reaction of grapevine rootstocks to *Pratylenchus brachyurus* and *Pratylenchus zaei*. *Tropic. Plant Pathol.* 37, 220–222.
- Resende, M.D.V. (2000). Análise estatística de modelos mistos via REML/BLUP na experimentação em melhoramento de plantas perenes (Colombo: Embrapa Florestas).
- Resende, M.D.V., Ramalho, M.A.P., Carneiro, P.C.S., Carneiro, J.E.S., Batista, L.G., and Gois, I.B. (2016). Selection index with parents, populations, progenies and generations effects in autogamous plant breeding. *Crop Sci.* 56, 530–546. <https://doi.org/10.2135/cropsci2015.05.0303>.
- Santos, E.A., Viana, A.P., Freitas, J.C.O., Rodrigues, D.L., Tavares, R.F., Paiva, C.L., and Souza, M.M. (2015). Genotype selection by REML/BLUP methodology in a segregating population from an interspecific *Passiflora* spp. crossing. *Euphytica* 204, 1–11. <https://doi.org/10.1007/s10681-015-1367-6>.
- Santos, P.R., Preisigke, S.C., Viana, A.P., Cavalcante, N.R., Sousa, C.M.B., and Amaral Junior, A.T. (2017). Associations between vegetative and production traits in guava tree full-sib progenies. *Pesq. Agropec. Brasil.* 62, 303–310. <https://doi.org/10.1590/S0100204X2017000500003>.
- Schuck, M.R., Biasi, L.A., Mariano, A.M., Lipski, B., Riaz, S., and Walker, M.A. (2011). Obtaining interspecific hybrids, and molecular analysis by microsatellite markers in grapevine. *Pesq. Agropec. Brasil.* 46, 1480–1488. <https://doi.org/10.1590/S0100204X2011001100009>.
- Silva, S.N., Silva, M.A., Marçal, T.S., Ferreira, A., Fontes, M.M.P., and Ferreira, M.F.S. (2017). Genetic parameters of pollen viability in guava (*Psidium guajava* L.). *Austral. J. Crop Sci.* 11, 1–8. <https://doi.org/10.21475/ajcs.2017.11.01.PNE91>.
- Starr, J.L., Cook, R., and Bridge, J. (2002). Plant Resistance to Parasitic Nematodes (Wallingford: CABI Publishing). <https://doi.org/10.1079/9780851994666.0000>.
- Taylor, A.L. (1967). Introduction to Research on Plant Nematology: An FAO Guide to Study and Control of the Plantparasitic Nematodes (Rome: Food and Agricultural Organization of the United Nations), 133 pp.
- Vencovsky, R., and Barriga, P. (1992). Genética biométrica no fitomelhoramento. (Ribeirão Preto: Sociedade Brasileira de Genética), 496 pp.
- Viana, A.P., and Resende, M.D.V. (2014). Genética Quantitativa no Melhoramento de Fruteiras (Rio de Janeiro: Interciência), 232 pp.
- Zasada, I.A., Riga, R., Pinkerton, J.N., Wilson, J.H., and Schreiner, R.P. (2012). Plant-parasitic nematodes associated with grapevines, *Vitis vinifera*, in Washington and Idaho. *Am. J. Enol. Vitic.* 63, 4. <https://doi.org/10.5344/ajev.2012.12062>.

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