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Development of rootstocks grapevine and cold stratification time

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Abstract

With the growth of new areas of grapevine planting in Brazil and other tropical countries, the rootstocks play an important role in the implantation of new areas and adaptation of cultivars. The development of new materials has increased productivity per area and even the final quality of the grapes. One of the problems in the propagation of rootstocks grapevine is the natural dormancy present in several species. We aimed to assess the stratification time's initial development and feasibility of grapevines rootstocks stem cuttings on field conditions. Two experiments were carried out. In the experiment of initial development of rootstocks grapevine, were used varieties: 'IAC 572', 'IAC 766', 'IAC 571-6', 'IAC 313', '420-A', '*Vitis Cinerea*', 'Riparia de Traviú', 'Rupestris du Lot', 'Kober 5BB', 'SO4', '8B', 'RR101-14', '99R', 'Schwartzmann' and 'Freedom'. In the experiment of stratification time feasibility of grapevines rootstock the varieties used were: 'IAC 572', 'IAC 766', 'IAC 571-6' and 'IAC 313'. In both experiments we assessed the stem cuttings' rooting, sprouting and roots and shoot development. Both experiments, we performed them in a randomized block experimental design, with four replications, ten stems per experimental plot, having in between these cuttings spaces of 12 x 4 cm. During the first experiment, the treatments consisted of 15 rootstock cultivars, and during the second experiment, we used a 4 x 3 factorial scheme, represented by 4 rootstocks and 3 stratification times. The variables we assessed were: percentage of rooted and sprouted cuttings, the branch's and root's length, number of leaves and branches, the roots' and total aerial dry mass. Among the assessed rootstocks in first experiment, the IACs rootstocks presented greater shoot vigor and the 'Riparia de Traviú' increased root system development during field settling. In the second experiment, the 'IAC 572' rootstock presented the best field development rates after stratification. The stem cuttings presented greater vigor when stratified cold during 20 days.

Keywords: propagation; root development; rootstocks; *Vitis* spp; vigor.

Introduction

The main method of woody fruit species' asexual multiplication is cuttings, because of its rooting quality and easiness in handling, in addition to making it possible to obtain uniform and identical clones to the original plant, facilitating nutritional, phytosanitary and cultural handling in the field. In Brazil, usually, vineyards are formed by planting woody cuttings directly into the field or by rooting them in plastic containers for a later planting in the field, and, in both cases, the cuttings remain in the field until they reach a minimum base diameter of 6.5 mm, with posterior grafting of the canopy cultivar (Regina et al., 1998; Gomes et al, 2002).

In modern viticulture, the use of rootstocks is a mandatory practice, with the purpose of offering resistance to phylloxera (*Dactilisfaerea viticola*), ground-pearl (*Eurhizococus brasiliensis*) and nematodes, (Pinkerton et al., 2005; Korosi et al. Al., 2011). In addition to the phytosanitary aspect, other characteristics are targeted with the use of rootstocks, such as the adaptation to acidic soils (Salino et al., 2006; Villa et al., 2009) and saline (Viana et al., 2001; Desouky et al. , 2005), as well as the grape's vigor, production and quality (Mota et al., 2009; Pavellok, 2011; Rizk-Alla et al., 2011).

Due to the expansion of grapevine cultivars in tropical climate regions, such as in São Paulo's Northwest State region and in the São Francisco Valley, the rootstocks 'IAC 572', 'IAC 766' and 'IAC 313', regarding vigor, compatibility with several canopy varieties and adaptability in various types of soils, had an important contribution to the development of national viticulture practices. In addition to the cultivars developed by the Agronomic Institute, there are numerous cultivars of rootstocks traditionally used in national and worldwide viticulture. Studies have shown that rootstocks with a high percentage of rooting and cuttings' sprouting, along with vigor during early field stages development, are the main criteria in choosing the best cultivar for the formation of commercial areas (Silva et al., 2010; Zhang, et al., 2016).

The use of the stratification technique for cuttings has the purpose of allowing a rest period to overcome endodormity, especially in tropical climate regions where the number of cold hours the grapevines take to sprout is less than or equal to zero, besides the quality maintenance of cuttings and the possibility of scheduling when to plant or graft (Regina, 2002).

During stratification, the content of rooting inhibiting substances decreases, increasing the auxin production capacity by the buds, providing this natural hormone downward polar movement to the cuttings' base, thus intensifying auxin activity in this region, favoring the root's early stages development (Pires and Biasi, 2003). It should be noted that, in the eastern and southwestern regions of the state of São Paulo, the conventional method of grapevines propagation used by wine producers consists of the staking grapevine cuttings for the early stages of root development for about 40 days, afterwards, the planting of cuttings in the field is performed, and the grafting is carried out during the winter of the following year. Thus, considering the commercial production, the cuttings storage in refrigerated conditions (cold stratification) also has the advantage of avoiding possible plant material contaminations in soils contaminated with pests or diseases. In the region of Jundiaí in 2012, it was reported that a Niagara Rosada vineyard cultivar grafted on the 'IAC 766' rootstock, presented the occurrence of the soil fungus *Phaeomoniella chlamydospora*, causing Petri disease in the grapevines (Ferreira et al., 2015), a recent, recurring and worrying problem in this wine-growing area.

Within this context, we aimed to assess the rooting, sprouting and development of the aerial part and the roots of different varieties and species of rootstocks, and, the feasibility of stratification time in sprouting and rooting of rootstocks stem cuttings of grapevines under field conditions.

Results and Discussion

Experiment 1: Initial development of grapevines rootstocks propagated by stem cuttings

Percentage of rooted, sprouted cuttings and length of the branch

There was no significant difference on the percentage of rooted and sprouted cuttings, with average values of 87.4 and 85.7%, respectively (Table 1). The assessed rootstocks did not present difficulties in rooting when propagated by stem cuttings, a characteristic inherited from their progenitors *Vitis riparia* and *Vitis rupestris*, which root easily, providing a vigorous budding (Pires and Biasi, 2003; Roberto et al., 2008).

The greatest length of the branch was obtained by the 'IAC 572' rootstock, with a value of 53.1 cm, followed by the rootstocks 'IAC 313', 'IAC 571-6' and 'IAC 766', with respective values of, 38.2, 37.1 and 24.3 cm, which differed significantly from the other rootstocks, evidencing the high vegetative vigor of cultivars generally called "Tropical". The 'Riparia de Traviú' rootstock presented the highest root length, 36.4 cm, although it did not differ significantly from 'IAC 571-6', 'IAC 572' and 'IAC 766' rootstocks, which presented average values of 27.9, 25.5 and 23, 8 cm, respectively.

In addition to the root development of a rootstock, the cuttings sprouting ability is also important, as these factors are closely related, once, under field conditions the rapid formation of new grapevines depends on the rootstocks vigor, in which, after one year of formation, a canopy variety will be grafted (Pires and Biasi, 2003).

Assessing 17 rootstocks vegetative performance, nine months after sowing in the field, Silva et al. (2010) concluded that 'IAC 572' showed a higher development, prior to grafting, however, a greater 'BRS Violet' shoots vigor was obtained when grafted on 'SO4', 'Harmony', 'Paulsen', 'IAC 766' and 'IAC 313'. The results confirm that the initial vigor of 'Tropical' rootstocks, even when staked, is reflected on their development and placement on the field.

Number of leaves and branches

The 'IAC 571-6' rootstock presented the highest number of leaves, with an average value of 15.4 leaves per cutting, although it hasn't significantly differed from the 'IAC 572', 'IAC 766', 'IAC 313', 'RR 101-14' and 'Riparia de Traviú' rootstocks. Regarding the number of branches, the highest value we obtained was from the 'RR101-14' rootstock, with 1.8 branches per cutting, significantly differing only from the 'Riparia de Traviú', 'Kober 5BB' and 'Freedom' rootstocks. The number of leaves and branches expresses the vigorous rootstock's adhesion capacity in the field, guaranteeing an photo-assimilated independent elaboration so its metabolism is no longer dependent on the reserves present in the cuttings (Taiz and Zeiger, 2013).

Dry mass

The highest dry mass of the roots we obtained was in the 'Riparia de Traviú' rootstock, 1.26 g, not significantly far from the rootstocks 'IAC 572', 'IAC 571-6', '420-A', 'Rupestris Du Lot' and 'Schwartzmann'. The 'IAC 572' and 'IAC 571-6' rootstocks presented the highest leaves dry mass values, 7.82 and 5.80 g, respectively, and total dry mass as well, 8.45 and 6.40 g , respectively. Studies with different rootstocks performed by Alvarenga and Fortes (1976) and Tecchio et al. (2007) also concluded that the 'IAC 572' rootstock had higher aerial dry mass values, reflecting the vigorous cuttings aerial development.

Experiment 2: Stratification time feasibility in the initial development of grapevines rootstock propagated by stem cuttings.

Percentage of rooted, sprouted cuttings and length of the branch

There was no significant interaction between the stratification time and the grapevines rootstocks regarding all characteristics we assessed (Table 2).

By analyzing the isolated effect of rootstocks, we could verify that the 'IAC 572' presented the highest percentage values of rooted and sprouted cuttings, with average values of 91.7% for both variables. However, these values differed, significantly, only from the 'IAC 766' rootstock, which presented 75.0% of rooted cuttings and 76.7% of sprouted cuttings. There was no significant stratification effect on the percentage of rooted and sprouted cuttings. According to Pires and Biasi (2003), the cold stratification consists of a dormancy overcoming process and an increase on cuttings rooting, through the decay of rhizogenesis inhibiting substances and, mainly, the increase on the hormone auxin activity, which we obtained in the current study.

Table 1. Percentage of rooted and sprouted cuttings, length of the largest shoot and the largest root, total number of leaves and shoots per cutting and dry mass of leaves, roots and branches from different rootstocks. Botucatu-SP, 2015.

Rootstock	Cuttings %*		Length (cm)		Number		Dry mass (g cutting^{-1})		
	Rooted	Sprouted	Shoot	Root	Leaves	Shoots	Aerial	Roots	Total
'IAC 572'	95.0 ns	95.0 ns	53.1 a	25.5 abc	20.8 ab	1.5 ab	7.82 a	0.64 ab	8.45 a
'IAC 766'	80.0	80.0	24.3 b	23.8 abc	15.6 abc	1.3 ab	2.74 bc	0.46 b	3.2 bc
'IAC 571-6'	90.0	92.5	37.1 b	27.9 ab	21.4 a	1.7 ab	5.80 ab	0.60 ab	6.4 ab
'IAC 313'	82.5	82.5	38.2 b	16.3 bcd	15.5 abc	1.1 ab	3.33 bc	0.27 b	3.6 bc
'420-A'	77.5	77.5	13.5 c	18.4 bcd	9.1 c	1.1 ab	1.81 c	0.56 ab	2.37 c
'*Vitis cinerea*'	70.0	70.0	17.4 c	13.7 cd	7.7 c	1.1 ab	1.11 c	0.12 b	1.23 c
'Riparia de Traviú'	90.0	90.0	20.3 c	36.4 a	11.8 abc	1.0 b	1.93 c	1.26 a	3.2 bc
'Rupestris du Lot'	90.0	92.5	14.8 c	15.3 bcd	10.4 c	1.3 ab	1.14 c	0.64 ab	1.78 c
'Kober 5BB'	90.0	85.0	15.5 c	10.0 d	6.2 c	1.0 b	0.86 c	0.07 b	0.93 c
'SO4'	97.5	95.0	11.3 c	9.7 d	6.0 c	1.2 ab	0.80 c	0.09 b	0.89 c
'8B'	80.0	80.0	10.5 c	17.6 bcd	8.2 c	1.1 ab	1.21 c	0.26 b	1.47 c
'RR101-14'	97.5	97.5	21.9 c	17.5 bcd	14.9 abc	1.8 a	2.25 c	0.40 b	2.65 c
'99R'	92.5	80.0	11.3 c	12.9 cd	7.4 c	1.2 ab	0.68 c	0.15 b	0.83 c
'Schwartzmann'	93.3	93.3	12.3 c	19.8 bcd	10.4 c	1.4 ab	1.65 c	0.55 ab	2.11 c
'Freedom'	85.0	75.0	12.3 c	18.4 bcd	11.1 bc	1.0 b	0.59 c	0.32 b	0.91 c
CV (%)	18.57	21.05	26.97	28.02	34.29	23.84	55.40	70.65	50.84
DMS	15.39	17.06	4.36	13.47	10.27	7.56	3.16	0.76	3.45

Average values followed by the same letter in the column do not differ from each other by the Tukey test at 5% probability. ns = not significant. * Transformed data (arc sin √x / 100).

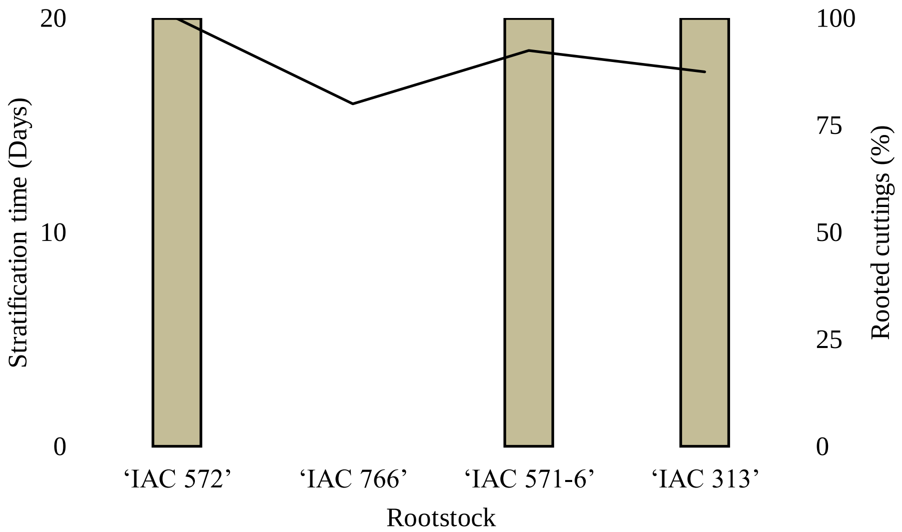

Fig 1. Stratification time and percentage of rooted cuttings of rootstocks.

Table 2. Percentage (%) of rooted and sprouted cuttings, length (cm) of the largest shoot and largest root, total number of leaves and shoots, total number (cutting^{-1}) of leaves and shoots, roots and total (aerial + roots) aerial (shoots + leaves) dry mass (g cutting^{-1}) of different rootstocks and stratification times. Botucatu-SP, 2015.

Rootstock	Cuttings %*		Length (cm)		Number (cutting^{-1})		Dry mass (g cutting^{-1})		
	Rooted	Sprouted	Shoot	Root	Leaves	Shoots	Aerial	Roots	Total
'IAC 572'	91.7 a	91.7 a	53.5 a	23.6 ab	18.5 ns	1.5 ns	7.33 a	0.69 ab	8.02 a
'IAC 766'	75.0 b	76.7 b	27.1 b	21.1 ab	13.8	1.3	3.01 b	0.80 a	3.82 b
'IAC 571-6'	88.3 ab	88.3 ab	37.4 b	26.0 a	16.4	1.5	5.17 ab	0.72 ab	5.89ab
'IAC 313'	81.7 ab	81.7 ab	37.1 b	18.2 b	13.5	1.2	3.57 b	0.39 b	3.86 b
Stratification (days)									
0	86.9 ns	87.5 ns	38.2 b	23.3 ns	18.3 a	1.4 ns	4.92 ab	0.49 b	5.41ab
10	76.9	77.5	30.3 b	19,0	12.6 b	1.2	3.41 b	0.51 b	3.92 b
20	88.8	88.8	47.9 a	24.3	15.7 ab	1.5	5.91 a	0.95 a	6.86 a
CV%	19.93	20.15	26.91	29.39	34.09	22.55	41.56	51.72	40.50
DMS	14.27	16.28	11.53	7.20	5.86	3.40	2.18	0.37	2.41

Average values followed by the same letter in the column do not differ from each other by the Tukey test at 5% probability. ns = not significant. * Transformed data (arc sin √x / 100).

When assessing stem cuttings of grapevines rootstocks under field conditions without previous treatment, Tecchio et al. ('IAC 572' and 'IAC 571-6') obtained average rooting values of 97%, 88% and 82% for the 'IAC 766', 'IAC 572' and 'IAC 571-6' rootstocks, respectively, regarding the percentage of sprouted cuttings, 87%, 79% and 76% were found, these values are similar to those we obtained in the present study, however, the 'IAC 572' rootstock presented the highest averages when compared to 'IAC 766'. Bordin et al. (2005) obtained, with semi stem cuttings, 83% rooting for the 'IAC 572' rootstock and 45% for the 'IAC 766', the rooting percentage for the 'IAC 572' rootstock, when compared to the results in the present study, was inferior, as well as the results obtained for the 'IAC 766' were.

The rootstocks developed by the IAC do not present difficulties in rooting, when propagated by stem cuttings, this property being inherited from their progenitors, grape species from tropical America, which present high vegetative vigor, good rooting and sprouting rates (IAC, 2001; Tecchio et al., 2007).

Time stratification

There was no stratification time effect on the percentage of rooted and sprouted cuttings. When working with water stratification, Roberto et al. (2008) concluded that stem cuttings from rootstocks subjected to stratification for 48 hours prior to planting presented better performance regarding root and shoot emission. Maroli et al. (2014) verified that stratification at temperatures of 19ºC and 24ºC, along with the application of IBA (2,000 mg L^{-1}) after stratification, favored the plants survival of the 'Bordô' cultivar on the 'Paulsen 1103' rootstock, obtained by table grafting. However, we can notice the positive effect of different stratification types on the development of grapevine rootstock cuttings, and it is not different with cold stratification. The best stratification times for the rooting of grapevine rootstocks are shown in graph 1.

Shoot and root length

The 'IAC 572' rootstock had the longest shoot length, with an average value of 53.5 cm. The cuttings shoot length kept stratified for 20 days in the cold was of 47.9 cm, a statistically higher result than those stratified at 0 and 10 days, with 38.2 and 30.3, respectively.

Regarding roots length, the 'IAC 571-6' rootstock had the highest value, 26 cm, differing only from the 'IAC 313', with 18.2 cm. There was no significant stratification time effect on rootstocks roots length. There was, however, a significant stratification time effect on the rootstocks shoots length, and the cuttings that were stored for 20 days presented higher average values, with 47.9 cm. These data do not agree with those obtained by Salibe et al. (2010), which assessed cold-stratified cuttings from 'VR 043-43' rootstocks, and obtained longer roots when compared to non-stratified cuttings and without IBA application, conferring a higher cuttings' root dry mass.

The length of the branches and the root system are parameters that determine the initial vegetative vigor of rootstocks, allowing them to predict their attaching capacity and also a fast development in the field which, later, are grafted with the canopy cultivar (Pires and Biasi, 2003).

Shoot dry mass and total dry mass

The 'IAC 572' rootstock presented the highest values of shoot dry mass and total dry mass, differing significantly from the values found on the 'IAC 766' and 'IAC 313' rootstocks under the same circumstances. Tecchio et al. (2007) verified values in the aerial dry mass of 'IAC 571-6' and 'IAC 572' rootstocks higher than those obtained in the present study, the same happened for the root's dry mass. Bordin et al. (2005) did not find any difference on the roots' dry mass of 'IAC 572' and 'IAC 766' rootstocks, in cuttings with absence of leaves, but they observed that the 'IAC 572' exceeded the 'IAC 766' regarding cutting treatments with whole and half leaves. Regarding the dry mass of roots, there was no significant difference between 'IAC 766', 'IAC 572' and 'IAC 571-6' rootstocks.

The highest values of shoot dry mass and total dry mass we obtained at 20 and 0 days of stratification, presenting 5.91 and 4.92 g of aerial dry mass and 6.68 and 5.41 g of total dry mass, respectively. Similarly, cuttings that were kept in stratification for 20 days had higher values of root dry mass, with 0.95 g.

Number of leaves

The increase on the cuttings shoots length and number of leaves is due to the stratification time, which induces the decay of compounds that inhibit the action of indoleacetic acid, a naturally occurring auxin in plants, a hormone responsible for the natural emission of roots in cuttings, partially, a well-developed root system adds vigor to the plants canopy, evidenced mainly by the size of the branch and leaves' characteristics, as well as their number (Hartmann et al., 2002; Taiz and Zeiger, 2013).

There was no significant difference between the rootstocks regarding the number of leaves and shoots per cutting. Few are the reports on the number of leaves and shoots per cutting found in literature, both of which show that rootstocks have similar vigor characteristics under field cultivation conditions. However, the number of leaves was higher in cuttings that hadn't undergone the stratification process, presenting 18.3 leaves, although it differed significantly from those that were stratified for 10 days.

Materials and Methods

Experimental location and plant materials

We carried out the experiment at the Experimental Orchard of the Horticulture Department, Agronomic Sciences College, Unesp, Botucatu, SP, at 22 ° 52 ' 20 " S, 48 ° 26 ' 37 " W and 840 m altitude. The climate classification, according to Köppen, is of the Cwa type, that is, a hot temperate climate with dry season coinciding with the winter and minimum average annual temperature of 15.3 ° C and maximum of 26.1 ° C, with an annual rainfall rate of 1.360 mm (Cepagri, 2016).

Two experiments were carried out. In both of them, we obtained rootstocks cuttings from 3-year-old nursery plants, spaced at 2.0 x 1.0 m and kept on a high-strap system with unilateral cord. Leafless stem cuttings with an average diameter of 6 mm and a length of 35 cm were used, with 4 to 5 buds per cutting, which were collected on July 6th, 2015. We prepared the cuttings with a basal 0.5 cm cut below the first bud, and a top bevel 3 cm cut above the last bud.

The soil of the experimental area was classified as Red Nitosol according to Embrapa criteria (1999), presenting the following chemical characteristics: pH (CaCl2): 5.14; organic matter: 21.8 g dm-3; Ca+2: 34.5 mmol dm-3; Mg+2: 11.4 mmolc dm-3; K+: 1.34 mmol dm-3; H+ + Al+3: 36.9 mmolc dm-3; base sum: 47.3 mmol dm-3; T: 84.2 mmolc dm-3 and base saturation: 56.3%. We performed manual irrigation on alternate days in order to keep the soil in field capacity.

Experimental design and treatments

In the 1st experiment, we used an experimental design in randomized blocks with 15 treatments, 4 blocks and experimental plot containing ten cuttings from each rootstock cultivar. The evaluated rootstocks and their origin are shown in Table 3.

Table 3. Rootstocks and their origin evaluated in the experiments.

Rootstocks	Origin
'IAC 572'	*Vitis tiliaefolia* x *V. riparia* x *V. rupestres*
'IAC 766'	'Ripária de Traviú' x *V. caribaea*
'IAC 571-6'	*V. caribaea* x 'Pirovano 57'
'IAC 313'	'Golia' x *V. cinerea*
'420-A'	*V. berlandieri* x *V. riparia*
Vitis cinerea	*V. cinerea*
'Riparia de Traviú'	*V. riparia* x [*V. rupestris* x *V. cordifolia*]
'Rupestris du Lot'	*V. rupestris*
'Kober 5BB'	*V. berlandieri* x *V. riparia*
'SO4'	*V. berlandrei* x *V. riparia*
'8B'	*V. berlandieri* x *V. riparia*
'RR101-14'	*V. riparia* x *V. rupestres*
'99R'	*V. berlandrei* x *V. rupestres*
'Schwartzmann'	*V. riparia* x *V. rupestris*
'Freedom'	'Dog Ridge' x 'Couderc 163'

In the 2nd experiment, we used an experimental design in randomized blocks on a 4 x 3 factorial scheme, each of them corresponding to one of the four rootstocks ('IAC 572', 'IAC 766', 'IAC 571-6' and 'IAC 313') and three stratification times (0, 10 and 20 days) in 4 blocks, containing ten stakes of each rootstock per plot. The rootstocks stem cuttings were separated into three batches, which were subjected to cold stratification times in 0 (no stratification), 10 and 20 days. The rootstocks cuttings we did not submit to stratification were planted soon after their preparation. Cuttings submitted to cold stratification for 10 and 20 days were stored in a cold room at 5 ± 1 ° C and 85 ± 5 % relative humidity, wrapped in moistened paper and packed in a plastic bag (Regina, 2002).

We planted cuttings from both experiments in a bed of 0.25 x 1 x 30 m in height, width and length, respectively, covered with a 30% shading screen 2 m from the ground, with 10 x 15 cm spaces between the cuttings. The planting was carried out keeping 2/3 of the cuttings buried.

Characteristics evaluated

In both experiments, the following variables were assessed: percentage of rooted and sprouted cuttings by visual counting of shoots and roots; the length of the main branch (cm) and main root (cm), determined with the aid of a graded ruler; number of leaves and branches per cutting, considering fully expanded leaves and branches over 15 cm in length; the dry masses from the aerial (g), roots dry mass (g) and total dry mass, determined from the sum of the dry masses from the aerial part and the roots. In order to obtain the dry mass, the materials were deposited in an intermittent flow greenhouse at 65 ° C until reaching a constant mass, and weighed with an analytical balance.

Statistical analysis

We submitted the data to analysis of variance and the averages were compared by the Tukey test at a 5% probability, using the SISVAR statistical program (Ferreira, 2011). The data transformation was used for the percentage of rooted and sprouted cuttings, because they did not present normal distribution, using the formula arc sin √x / 100.

Conclusion

The 'IAC 572' rootstock showed greater vigor of the aerial part and 'Riparia de Traviú', 'IAC 571-6', 'IAC 572' and 'IAC 766' greater development of the root system in field establishment. The 'IAC 572' rootstock presented the best development rates in the field when stratified cold in 20 days. Cold stratification in 20 days is recommended for the development of grapevine rootstocks in the field.

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