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Effect of quick-dip with increasing doses of IBA on rooting of five grapevine rootstocks grafted with 'Cabernet Sauvignon'

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Summary

The establishment of a vineyard depends on clonal propagation of grapevine woody cuttings. The successful plant propagation relies on adventitious root formation, but it is affected by the scion-rootstock interaction. Synthetic auxins are used in the nursery to improve rooting of woody cuttings. We tested the effect of a quick dip (30 seconds) of increasing doses of indole-3-butyric acid (IBA) solutions (0, 500, 750, 1000, 1250, 1500 ppm) on rooting of grafted woody cuttings. We tested 5 rootstocks grafted with *V. vinifera* 'Cabernet Sauvignon': SO4 (*V. berlandieri* x *V. riparia*), 110 Richter and 1103 Paulsen (*V. berlandieri* x *V. rupestris*), 101-14 Mgt (*V. riparia* x *V. rupestris*) and Cereza (*V. vinifera* L.). After 21 days in the callus chamber, we examined the quality of the root system by means of different rooting parameters (callusing and rooting percentage and number, length and biomass of roots per cutting). Among all the rooting parameters evaluated, Cereza presented the highest values in relation to the rest of the rootstocks, followed by 101-14 Mgt, SO4, 110R and 1103P. The quality of the root system was improved in terms of number of roots, length and the total biomass on four rootstocks: Cereza, 110R, 101-14 Mgt and SO4, but not in 1103P whose rooting was unaffected by treatments. The optimal dose of IBA through quick-dip at improving rooting was rootstock-dependent, being 750 ppm for 110R, 101-14 Mgt and SO4; and 1000 ppm for Cereza. These results will contribute in reducing costs during clonal propagation in the nursery, which is associated with the use of costly synthetic hormones and a lower production of bare-root vines.

K e y w o r d s : *Vitis*; woody cuttings; propagation; adventitious roots formation.

Introduction

Grapevine (*Vitis vinifera* L.) is one of the most important fruit crops worldwide (FAOSTAT, 2021). The establishment of vineyards depends on clonal propagation of woody cuttings. The success of plant propagation relies on adventitious root formation originated from woody cuttings. In the nursery, woody cuttings are forced to produce adventitious roots in a callus chamber. Adventitious root formation is affected by external factors such as humidity, temperature and aeration, but is also regulated by a host of internal factors including auxins and cytokinin, carbon and nitrogen reserves, inhibitors, among other compounds, as well as by the genetic background of the cuttings. However, only auxins have been clearly identified as the main regulators for adventitious root formation (Smart *et al.* 2002, Hartman 2014, Keller 2020).

Grafting is widespread in Viticulture since rootstocks can induce resistance to soil-borne pests, diseases and enhanced tolerance to abiotic stresses (Hartman 2014, Assuncão *et al*. 2016, Ollat *et al.* 2016). *V. vinifera* cultivars are generally grafted onto rootstocks of American *Vitis* species and their hybrids. The three North American *Vitis* species that became widely used in rootstock breeding and crossbreeding programs were *V. berlandieri*, *V. riparia*, and *V. rupestris*. These rootstocks became relevant in the 19th century after the expansion of the devastating phylloxera (*Dactylosphaera vitifoliae*) given their resistance to this pest, among other characteristics (Legros 1993, Arnold *et al.* 2020).

A substantial number of American *Vitis* rootstocks are recalcitrant to form adventitious roots generating economic losses associated with unsuccessful propagation in the nursery. In particular, rootstocks or hybrid rootstocks of *V. berlandieri* are the most difficult to propagate because this species root poorly. The species *V. riparia* and *V. rupestris* can root more easily than *V. berlandieri* (Riaz *et al.* 2019). The classical and most widespread rootstocks in the world are four to five hybrids of these species: SO4 and 5BB from the *Berlandieri-Riparia* family; and 110R, 1103P, 140Ru from the *Berlandieri-Rupestris* family. The rootstock 101-14 Mgt (from the *Riparia-Rupestris* family) is the most used in the North of the USA and it is also important in Argentina (Ollat *et al.* 2016).

Auxin and auxin-like (such as IBA, indole-3-butyric acid) products have proven effective at increasing the rooting ability of such rootstocks, improving propagation success. There is some available information on adventitious root

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formation of some of these classical rootstocks with different IBA concentrations. Optimal auxin doses mediated by long-dip (24-48 h) (Murillo de Albuquerque *et al.* 2012, Dasalakis *et al.* 2019, Isçi *et al.* 2019) and quick-dip (from seconds to few minutes) treatments of IBA have been reported (Satisha *et al.* 2008). To minimize production times commercial grapevine nurseries prefer using quick-dip treatments of auxin.

Furthermore, it has also been reported that rooting of the cuttings could also be affected by the scion-rootstock interaction (Tandonnet *et al.* 2010, Ferlito *et al.* 2019 and local nursery communication). This is given because the scion-rootstock grafting union occurs at the same time as adventitious root formation in the callus chamber and it also depends on the above-mentioned internal factors of the scion and the rootstock woody cuttings.

Argentina is the fifth largest wine producer in the world (OIV 2021, INV 2021). In Argentinean Viticulture, *V. vinifera* Cereza (a native table grape variety) is also becoming relevant as a rootstock because it confers tolerance to salinity and nematodes (Di Filippo 2008; local nurseries websites). Therefore, the main aim of this work is to assess the effect of quick-dip with different doses of IBA on the rotting of the five most used rootstocks in Argentinean Viticulture. These are the four classical rootstocks: SO4, 110 Richter, 1103 Paulsen, 101-14 Mgt and Cereza (*Vitis vinifera*) grafted with 'Cabernet Sauvignon'. We hypothesize that the optimal concentration of IBA, inducing the major number of heavier and longer roots per cutting, is rootstock-dependent. We believe that this knowledge will assist the viticulture community at large, as well as local grapevine nursery farmers.

Material and Methods

The study was conducted in the nursery of Peñaflor Group S.A (Trapiche Winery). located in Santa Rosa, Mendoza, Argentina (33°15'39.4"S 68°07'48.9"W). Two-bud's woody cuttings of *V. vinifera* 'Cabernet Sauvignon' were grafted on 5-buds' woody cuttings of five rootstocks: SO4 (*V. berlandieri* × *V. riparia*), 110R and 1103P (*V. berlandieri* × *V. rupestris*), 101-14 Mgt (*V. riparia* × *V. rupestris*) and Cereza (*V. vinifera*). Further details of the rootstocks are presented in Tab. 1. A total of 900 cuttings (180 of each rootstock) were grafted using the omega cut with a bench grafting machine. The grafting union was coated with a commercial wax to avoid tissue dehydration and prevent fungus diseases.

Then, the 180 grafted cuttings of each rootstock were divided into 6 groups of 30 cuttings. The base (1.5 cm) of each group of cuttings (40 cm long) were dipped in six different doses (0, 500, 750, 1000, 1250 and 1500 ppm) of indole-3-butyric acid (IBA) solutions for 30 s. A total of 30 combinations of treatments were obtained. Grafted cuttings were placed horizontally in plastic boxes filled with a wet peat-based commercial substrate (KEKKILÄ professional, https://www.kekkilaprofessional.com/). After 21 d in the callus chamber (dark, 28 °C and 80 % relative humidity), the grafted cuttings were transferred to IBAM (Instituto de Biología Agrícola) for rooting parameters determinations.

To determine the effects of treatments on rooting and cutting roots quality, we measured percentage of callogenesis and percentage of rooting, as well as number of roots, root length (cm) and root biomass (g) per cutting. We measured the percentage of callogenesis by visual assessment of the callus covering the perimeter of the cutting base (0, 25, 50, 75 or 100 %). Root biomass per cutting was determined by weighing dried tissues (at 60 °C for 48 h) on an analytical scale. To determine root length, adventitious roots were excised using a scalpel and photographed on a black background. Then, root length was measured using ImageJ free software (https://imagej.nih.gov/).

The experiment had a completely randomized design, being the grafted cuttings, the experimental units. All statistical analysis was done with InfoStatP software 2020v (Di Rienzo *et al.* 2018). Rooting parameters of each rootstock were analyzed by fitting linear mixed effects models considering treatments (IBA doses) as a fixed factor (α = 0.05). Root biomass was fitted using a General Linear Model (GLM), whereas callogenesis percentage, rooting

Table 1

Rootstock characteristics (Hartman *et al.* 2014, Keller 2020)

Rootstocks	Name	Species	Procedence	Characteristics
Selection Oppenheim #4	SO4	*V. berlandieri* × *V. riparia*	American	Highly resistant to phylloxera and nematodes. Good performance on sandy soils.
110 Richter	110R	*V. berlandieri* cv. Rességuier × *V. rupestris* cv. Martin	American	Drought tolerance It's slightly more vigorous than the 1103P. Phylloxera resistant, but susceptible to nematodes. Limestone soil tolerant.
1103 Paulsen	1103P	*V. berlandieri* cv. Rességuier × *V. rupestris* cv. Du Lot	American	Phylloxera resistant Susceptible to nematodes. Drought tolerance and adapted to saline and compact soil.
101-14 Millardet et de Grasset	101-14 Mgt	*V. riparia* × *V. rupestris*	American	Resists phylloxera and many nematode species. Sensitive to water stress and acid soils. Performs best with adequate irrigation and on clay soils.
Cereza	Cereza	*V. vinifera* cv. Cereza	Euroasiatic	Phylloxera and salinity tolerance.

percentage, and number and length of roots per cutting were analyzed with Generalized Linear Mixed Models (GLMM) using a binomial distribution for the first two variables, and a Poisson distribution for the remaining parameter. Differences between means were assessed by a Fisher's LSD post hoc test.

Results

The percentage of callogenesis was greater than 75 % (that is the minimum required by the nursery to plant the already rooted cuttings at high densities in the field) in all the assessed rootstocks. We did not find differences among IBA-doses treatments ($P > 0.05$, data not shown), which means the callogenesis process was promoted in the callus chamber regardless of auxin concentration used.

IBA treatments higher than 500 ppm doubled the rooting percentage (30 vs 65-70 %) of SO4 rootstock (Fig. 1 A). All IBA doses increased three times the number of roots per cutting (Fig. 1 B), but the root length was unaffected by IBA treatments (Fig. 1 C). Root biomass per cutting was doubled with all IBA doses (Fig. 1 D). Therefore, for SO4 rootstock, the optimum concentration of IBA through quick-dipping was 750 ppm.

The rooting percentage of 110R was even lower than in SO4 (40 vs. 80 % with > 500 ppm). The doses between 500 and 1250 ppm of IBA increased the rooting percentage (40 %) with respect to the control (10 %) (Fig. 2 A). Doses between 500 and 1000 ppm of IBA increased the number and root biomass per cutting (Fig. 2 B and D). However, root length was similar among treatments and it was not affected by IBA doses (Fig. 2 C). In this rootstock, intermediate concentrations of IBA increased rooting percentage, number and

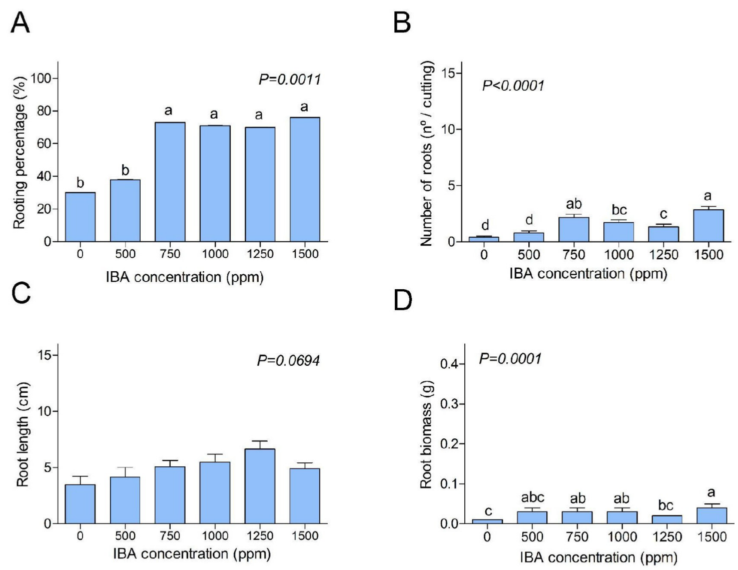

Fig. 1: SO4 rootstock. **A**) Rooting percentage, **B**) number, **C**) length and **D**) root biomass per cutting. Values are adjusted means ± EE, n = 30. Data were analyzed with GLM and GLMM. Different letters indicate significant differences between treatments according to the LSD Fisher post-hoc test (α = 0.05).

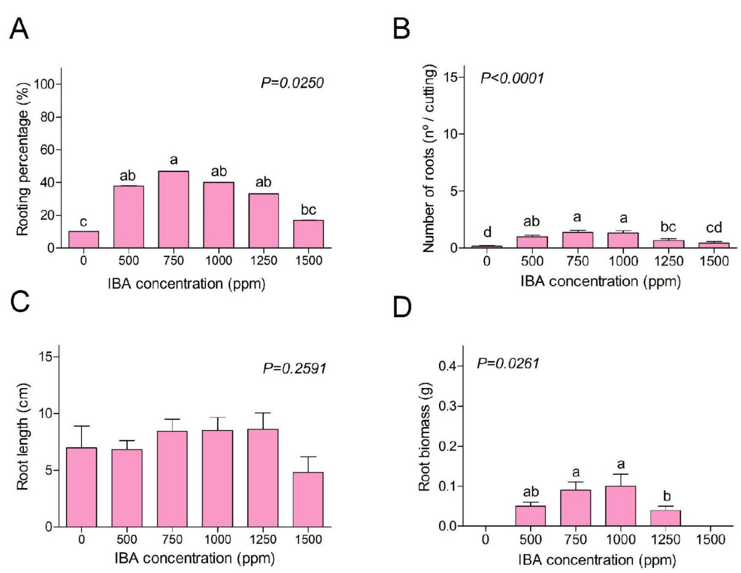

Fig. 2: 110R rootstock. **A**) Rooting percentage, **B**) number, **C**) length and **D**) root biomass per cutting. Values are adjusted means ± EE, n = 30. Data were analyzed with GLM and GLMM. Different letters indicate significant differences between treatments according to the LSD Fisher post-hoc test (α = 0.05).

root biomass per cutting, while high concentrations of IBA (1500 ppm) had an inhibitory effect on these parameters. The optimum concentration of IBA that enhanced the rooting of this rootstock was 750 ppm. IBA treatments did not affect the rooting percentage, nor the number and root biomass per cutting of 1103 P (Fig. 3 A, B and D). Concentrations higher than 750 ppm of IBA only increased root length by 20 % (Fig. 3 C). Consequently, we found that the rooting of 1103P is not promoted by IBA through quick-dipping.

Nor the rooting percentage or the root biomass per cutting of 101-14 Mgt rootstock was promoted by IBA treatments (Fig. 4 A and D). However, all IBA doses increased the number of roots per cutting compared to control (Fig 4 B). In addition, concentrations higher than 500 ppm increased root length (Fig. 3 C). Therefore, 750 ppm was the optimum concentration for improving the rooting of this rootstock.

The rooting percentage of Cereza rootstock was high in all the treatments (> 80 %), but this parameter was not affected by IBA-doses (Fig. 5 A). However, the number, length and root biomass were affected by increasing doses of IBA (Fig. 5 B, C, D). Concentrations higher than 750 ppm promoted the number of roots per cutting (Fig. 5 B). The longest roots were found at 1000 ppm (Fig. 5 C), while concentrations higher than 750 ppm increased root biomass per cutting (Fig. 5 D). Even though Cereza was rooted without IBA supplementation, the rooting of this rootstock (higher root length and biomass per cutting) could be improved by quick-dipping with 1000 ppm.

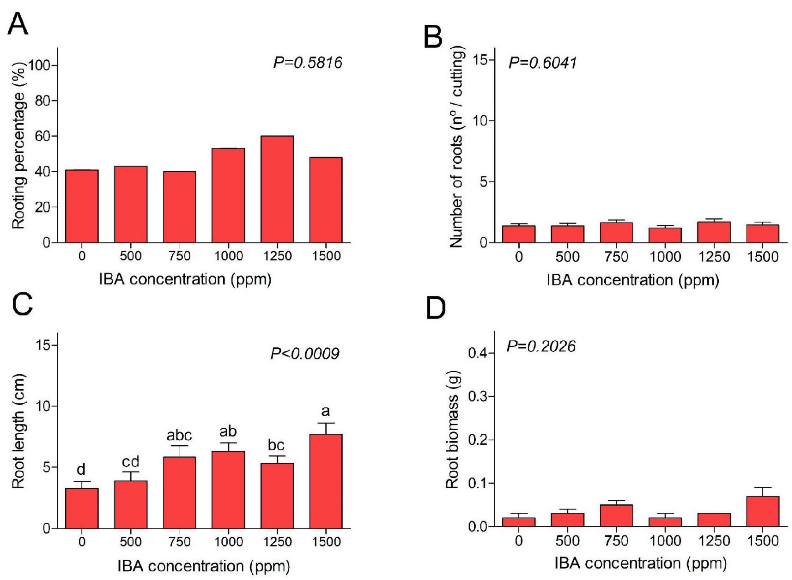

Fig. 3: 1103P rootstock. **A**) Rooting percentage, **B**) number, **C**) length and **D**) root biomass per cutting. Values are adjusted means ± EE, n = 30. Data were analyzed with GLM and GLMM. Different letters indicate significant differences between treatments according to the LSD Fisher post-hoc test (α = 0.05).

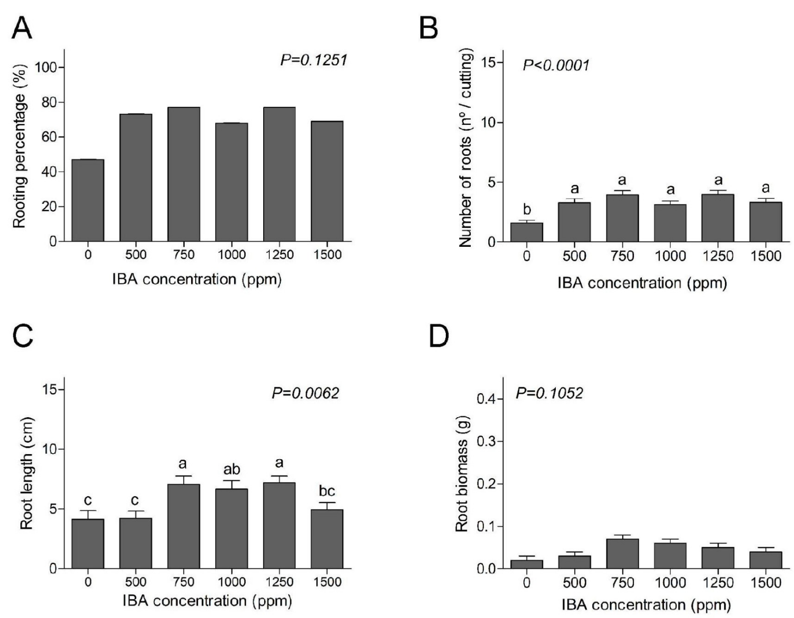

Fig. 4: 101-14 Mgt rootstock. **A**) Rooting percentage, **B**) number, **C**) length and **D**) root biomass per cutting. Values are adjusted means ± EE, n = 30. Data were analyzed with GLM and GLMM. Different letters indicate significant differences between treatments according to the LSD Fisher post-hoc test (α = 0.05).

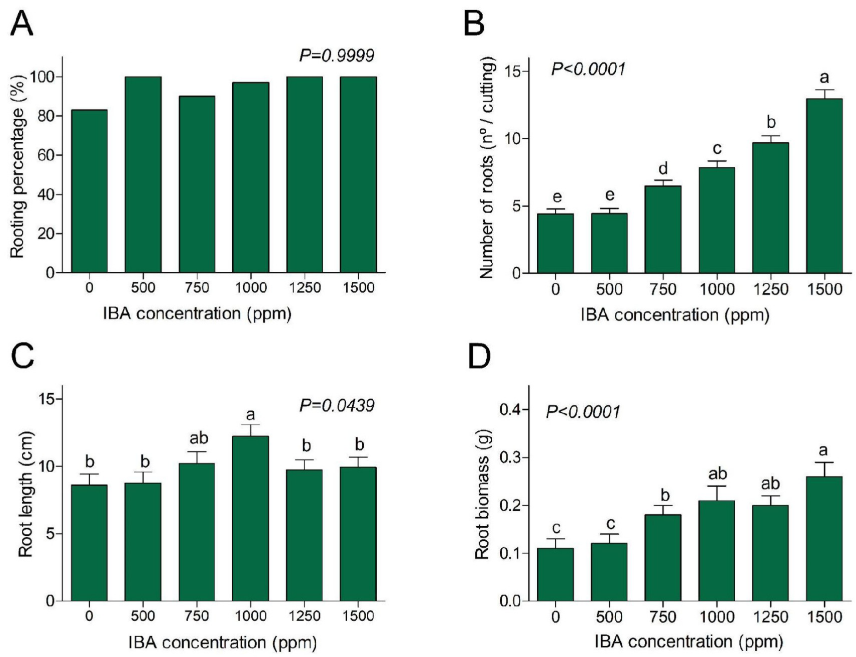

Fig. 5: Cereza rootstock. **A**) Rooting percentage, **B**) number, **C**) length and **D**) root biomass per cutting. Values are adjusted means ± EE, n=30. Data were analyzed with GLM and GLMM. Different letters indicate significant differences between treatments according to the LSD Fisher post-hoc test (α = 0.05).

Discussion

Given the capacity to form adventitious roots among the different rootstocks is very variable, and it is affected by the scion-rootstock interaction, it is important to know the optimum dose of synthetic auxins to be used in the nursery. In this work, we assessed the effect of increasing doses of IBA through quick-dipping on the rooting of the five grapevine rootstocks grafted with a widespread cultivar 'Cabernet Sauvignon'. These five rootstocks are the most relevant in Argentinean Viticulture. Of these rootstocks, four are the classical and most widespread rootstocks in the world: SO4 (Berlandieri-Riparia family), 110R, 1103P, (*Berlandieri-Rupestris* family) and 101-14 Mgt (*Riparia-Rupestris* family). The fifth rootstock is Cereza (*V. vinifera*) that is increasingly being used because it confers tolerance to salinity and nematodes. The rooting and the roots quality of the woody cuttings in response to increasing doses of IBA, were rootstock-dependent and variable between rootstocks. Even though Cereza is rooting without IBA supplementation, the rooting of this rootstock (in terms of higher root length and roots biomass per cutting) could be improved by IBA quick-dipping. The optimal dose of IBA through quick-dip at improving rooting was 750 ppm for 110R, 101-14 Mgt and SO4; and 1000 ppm for Cereza, while the rooting capacity of 1103P was unaffected by IBA-doses treatments (Fig. 1-5). The latter results are in agreement with a previous study, where a prolonged immersion (24 h) of 1103P woody cuttings in IBA solutions (0, 50, 100, 100, 200 and 400 ppm) was evaluated and none of the IBA treatments promoted the rooting of this rootstock (Murillo de Albuquerque *et al.* 2012). However, there are other studies reporting that IBA treatments promoted 1103P rooting (Satisha *et al.* 2008, Daskalakis *et al.* 2019). Even though these reports are contradictory, these differences may rely on other factors affecting the rooting process of 1103P, *i.e.*: type of substrate, inhibitors concentration, scion cultivar, etc. Some authors (Satisha *et al.* 2008) recommended 1000 ppm as the optimum IBA dose for quick-dipping for 1103P, 110R and SO4. As we already mentioned the scion-rootstock interaction affects rooting. In none of the above-mentioned reports the rootstock's cuttings were grafted with 'Cabernet Sauvignon'. To our knowledge this is the first study reporting optimal doses of IBA through quick-dipping for 101-14 Mgt and Cereza rootstocks.

Among all the rooting parameters evaluated (rooting percentage, number, length and root biomass per cutting), Cereza presented the highest values in relation to the rest of the rootstocks, followed by 101-14 Mgt, SO4, 110R and 1103P (Figs 1-5, Tab. 2). These results are in agreement with a previous study, where it was reported that rooting (measured as number of roots per cutting) capacity of Cereza was higher than SO4 and 1103 Paulsen (Di Filippo 2008). Although the supplementation of auxins was not evaluated, all the rootstocks were grafted with 'Malbec'. Moreover, SO4, 1103P and 110R were expected to have a lower rooting capacity than Cereza and 101-14 Mgt, given that these rootstocks are hybrids of *V. berlandieri*, which is a difficult–to-root American *Vitis* species (Riaz *et al.* 2019, Keller 2020).

In this study, we also found that quick-dipping of cuttings with high auxin concentrations inhibited adventitious root production in Cereza, 110R, 101-14 Mgt and SO4 rootstocks (Fig. 1, 2, 4 and 5). The optimum auxin dose for improving rooting was lower than the highest dose used (1500 ppm). Similar results were obtained on *V. vinifera* 'Thompson Seedless' grafted onto 1103P, 110R and SO4 (Satisha *et al.* 2008). In both studies, a quick-dip with 2000 ppm of IBA inhibited rooting percentage. As it was already mentioned, the genetic background of each species

Table 2

Rooting parameters at IBA optimal doses of the different rootstocks evaluated

Rootstock name	IBA dose	Rooting percentage (%)	Number of roots per cutting	Root length (cm)	Root biomass (g)
SO4	750 ppm	73	2.17	5.09	0.03
110R	750 ppm	47	1.37	8.46	0.09
1103P	-	40	1.6	5	0.03
101-14 Mgt	750 ppm	77	3.94	7.08	0.07
Cereza	1000 ppm	90	7.84	10.22	0.21

also affects the rooting capacity of the rootstocks (Smart *et al.* 2002). Another of the characteristics mentioned in literature about species that are more difficult to root is that the sclerenchyma ring, located between the vascular cylinder and the cortex outside the point of origin of the adventitious roots, is denser than in easy-rooting species (Hartmann *et al.* 2014). Sclereids are highly lignified cells, which support the stems and form a mechanical barrier that serves as a defense against pests. Species that are easier to root have fewer layers of cells in this ring, or have discontinuities in the ring. The same is true for cuttings with a larger diameter than recommended (10-12 mm), as they are longer-lived cuttings where the sclerenchyma has more cell layers (Hartmann *et al.* 2014). Unfortunately, we did not find reports comparing anatomical differences between the rootstocks assessed in this study and although the plant material used in this study was homogeneous, we did not measure the cuttings diameter to relate this parameter with the rooting parameters.

The information of the optimal doses of IBA through quick-dip promoting rooting of different rootstocks that is reported in this study, will contribute in reducing costs during clonal propagation in the nursery, which is associated with the use of costly synthetic hormones and a lower production of bare-root vines.

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