



A quantitative assessment of factors affecting the rooting of grapevine rootstocks (*Vitis vinifera* L.)

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ABSTRACT. Grapevine is a fruit species of great agronomic interest since both fresh fruit and wine are highly marketable products. Therefore, there is growing interest in this crop among researchers in the pursuit of increased yields and better cultivation conditions. Asexual propagation is the most commonly used method for propagating grapevine seedlings, with cuttings used for rootstock formation and subsequent grafting of materials to form the canopy. The rootstock is responsible for support, water, and nutrient uptake, thus determining the vigor of the plant; therefore, it is essential to understand the suitable conditions in which roots can thrive and thereby enhance plant growth. The type of cutting, type of substrate, and application of exogenous auxins are among the factors that interfere with the rooting of grapevine rootstocks, and determining and implementing the best combination of these factors could provide for more vigorous plants. Reviewing high-quality published scientific research from diverse countries is a method of identifying data, grouping information, and obtaining reliable answers. Furthermore, it can allow for the detection and grouping of the main factors affecting the rooting of grapevine cuttings, thus enabling a clearer recommendation and better understanding of the issue.

Keywords: auxin; seedling; substrate.

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Introduction

Grapevine (*Vitis vinifera* L.) is a species of the Vitaceae family, native to the Mediterranean region, Central Europe, Southwest Asia, Morocco, from northern Portugal to southern Germany, and eastern to northern Iran. The genus *Vitis* includes many species worldwide. Its diffusion and importance are highlighted by the diverse usage of grapes, including by the consumption of fresh and dried fruits (raisins), and the production of wines, juices, vinegar, ethanol, jams, fertilizers, and antioxidant compounds. In addition, the medicinal properties of the grape aid in the prevention of cancer, heart disease, allergies, and diabetes (Neto, Ricardino, Souza, Aguiar, & Marques, 2020).

To obtain quality fruit, attention to all stages of cultivation is paramount, and seedling production is considered crucial for the successful installation of a vineyard. The sexual propagation of grapevines results in large plant heterogeneity as well as late and uneven fruit sets (Fronza & Hamann, 2015). Therefore, the use of this technique is restricted to breeding programs (De Albuquerque & Choudhury, 1993; Villa et al., 2003). The rooting of cuttings is the most commonly used technique for producing grapevine seedlings (De Albuquerque & Choudhury, 1993), by using a small portion of the branch of a parent plant to produce a new plant by growing adventitious roots (Bettoni et al., 2014a). The cutting technique is widely used for the preservation of rootstocks (Fronza & Hamann, 2015) for subsequent grafting of a cultivar of interest and seedling production as the foundation of a vineyard (Regina, Souza, & Dias, 2012).

From a physiological and anatomical point of view, there is some complexity in root development by cuttings. Factors such as hormonal balance, cutting and substrate type (Nachtigal & Pereira, 2000), and hormone and plant growth regulators in the auxin group (Taiz & Zaiger, 2013; Vilarinho & Cândido, 2014) influence the dedifferentiation and redirection of totipotent cells for rooting.

The existence of qualitative and quantitative uncertainties of the aforementioned factors creates a demand for studies that present a clearer identification of the combinations that make the production of grapevine seedlings viable. Therefore, this study aimed to conduct a literature review on the main factors that affect the

rooting of grapevine cuttings and to provide a clearer and more objective recommendation for more successful seedling production.

In this literature review, research results on the factors that interfere with the rooting of grapevine cuttings were raised and discussed.

Material and methods

Data collection methodology

We reviewed 55 scientific articles published in journals with different impact factors and data from the most diverse grape-producing countries and regions. We chose to search for a better geographic representation of the information collected in order to cover as many particularities of each location as possible, thus reducing the effect of environmental variability on our review and conclusions. The databases used for the research were Scielo, Google Scholar, Science Direct, and CAPES websites.

Table 1 shows the number of articles selected for each investigation period (years) and the countries in which each study was conducted. Twenty-five (25) of the 55 articles were from the last ten years, of which 14 were from the past five years. Thirty-four (34) were from Brazil, and 21 were from eight other countries.

Table 1. Number of articles compiled over the past 40 years on the production of grapevine rootstock seedlings.

Year	No. of articles	Country	No. of articles
1980-1999	8	Brazil	34
		Egypt	1
		Greece	2
2000-2020	47	India	3
		Italy	4
		Palestine	1
Total	55	Pakistan	1
		Russia	1
		Turkey	8
		Total	55

The independent variables of the materials were grouped and defined as auxin dose (mg L^{-1}), immersion time (seconds or hour), cutting type, cutting length (cm), auxin, rootstock, substrate, and presence or absence of a leaf (Table 2). Rooting percentage was regarded as a dependent variable, and we considered the highest rooting percentage (%) of each article in this study (Table 3). A total of 926 rooting percentages were found, with the amount of data per paper varying from 1 (minimum) to 108 (maximum). Since large variability of the data was observed in most of the papers, the use of the average value could result in low rooting percentage values. In addition, using the highest value allowed us to obtain a combination of variables that result in better rooting of grapevine rootstock cuttings, which makes it possible to verify the trend of the results and simplify future decisions regarding new studies and/or practical applications that seek better results in the rooting of grapevine cuttings. Table 2 also shows the number of articles in which the results represent the levels of each variable.

Table 2. Distribution of the number of scientific articles and the variables that influence the production of grapevine rootstock seedlings.

No. of articles	Dose (mg L^{-1})
12	0
1	30
2	250
2	500
5	1000
1	1500
12	2000
4	3000
No. of articles	Auxin
31	IBA
1	IAA
1	NAA

No. of articles	Time (s)
12	5
12	10
3	15
3	20
3	24h
No. of articles	Rootstock
7	VR 043-43
3	IAC 572-Jales
6	Paulsen 1103
3	IAC 766
No. of articles	Substrate
7	Sand
6	Rice husk
8	Vermiculite
3	Soil
3	Nutrient solution
No. of articles	Cutting type
28	Hardwood
7	Semi-hardwood
12	Herbaceous
No. of articles	Cutting length (cm)
5	10
2	14
6	15
9	20
2	25
4	30
4	35
3	40
2	45
No. of articles	Leaf
2	0.3
5	0.5
8	1

Number (No.), indole-3-butyric acid (IBA), naphthalene acetic acid (NAA), and indole-3-acetic acid (IAA); leaf 0.3 (30% of a leaf), 0.5 (50% of a leaf), and 1 (100% of a leaf).

Table 3 shows the information required for the selected materials and relates them to our results. The variables were grouped in columns, and the main information was summarized and standardized to facilitate interpretation.

Table 3. Data compiled from 55 articles through the search items “grapevine × auxin”, “grapevine × cutting” and “grapevine × substrate.”

Rooting (%)	Dose (mg L ⁻¹)	Auxin*	Time (s)	Rootstock	Substrate	Cutting type	Cutting length (cm)	Leaf	Reference
100	1000	IAA	10	Crimson	Sandy soil	Hardwood	10		Ahmed et al. (2017)
100	2000	IBA	10	Paulsen 1103	Commercial	Hardwood	10	0.5	Amaral, Bini, and Martins (2009)
100	2000	IBA	5	IAC 766	Commercial	Semi-hardwood	-	1	Biasi, Pommer, and Pino (1997)
100	500	IBA	20	41B	Perlite	Hardwood	0		Gökbayrak, Dardeniz, Arıkan, and Kaplan (2010)
100	2000	IBA and IAA	20	41B-Italia	Sawdust	Hardwood	40		Köse and Güleriyüz (2006)
100				Ripária do Traviú, Kober 5BB, 420-A, IAC 572 'Jales'	Soil + sand + vermiculite + organic	Hardwood			Roberto, Pereira, Neves, Jubileu, and Azevedo (2004a)
100				Sultani Çekirdeksi, Buzgulu, Gokuzum and Siyah Dimrit	Nutrient solution	Hardwood	70		Sabir and Sabir (2018)
100	1000	IBA	5	101-14 e R. Du Lot	Sand	Hardwood	35		Da Silva, Fachinello, and Machado (1986)

100	3000	IBA	5	X1	Sand	Hardwood	25		Sozim and Ayub (2006)
99				IAC 572-Jales	Soil	Hardwood	25 and 9		Leão (2003)
99				RR101-14	Soil	Hardwood	30		Tecchio, Moura, Hernandez, Pio, and Wylar (2007)
98				IAC 766 'Campinas'	Rice husk	Herbaceous		0.5	Roberto et al. (2006)
97.5				Tropical (IAC-313)	Sand		40		De Albuquerque and Coudhury (1993)
96.8	1500	IBA	5	IAC 572-Jales	Rice husk		15	1	Faria et al. (2007)
96.3	3000	IBA	10	VR 043-43	Sand	Hardwood	35		Bettoni et al. (2014a)
96				775P	Peat + inert perlite	Hardwood			Bartolini, Carrozza, Scalabrelli, and Toffanin (2017)
95.8				Kober 5BB	Organic Nutrient solution	Hardwood	30		Monteguti et al. (2008)
95	250	IBA	24 h	Paulsen 1103		Hardwood	45		Daskalakis, Biniari, and Bouza (2019)
95	2000	IBA and IAA	5	140R and Kober 5BB	-	Hardwood	5		Kracke and Cristoferi (1985)
95				SO4	Vermiculite	Hardwood	20		Mayer, Biasi, and Bona (2006)
95				IAC 766	Rice husk	Herbaceous	20	1	Roberto, Kanai, and Yano (2004c)
95	2000	IBA	15	IAC 572 'Jales'	Commercial	Herbaceous	10		Souza, Lenk, Ono, and Rodrigues (2015)
94.6	3000	IBA	10	Muscat	Clay soil + Sand +and FYM	Hardwood	14		Chakraborty and Rajkumar (2018)
94				IAC 766 'Campinas'	Rice husk	Herbaceous	20	1	Roberto, Neves, Da Silva Jubileu, and De Azevedo (2004b)
92.6	2	IBA		Dog Ridge	Soil + sand + FYM	Herbaceous			Somkuwar, Bondage, Surange, and Ramteke (2011)
92.5				5 BB	Perlite	-			Dardeniz, Gökbayrak, Müftüoğlu, Türkmen, and BeŞer (2008)
92.5	0	IBA	10	VR 043-43	-	Semi-hardwood	10		Machado, Mayer, Ritter, and Biasi (2005)
92	0	IBA	10	VR 043-43	Commercial + Sand	Herbaceous	20	0.3	Botelho, Maia, Pires, Terra, and Schuck (2005b)
92	0	IBA	5	IAC 766	Sand	-	40	-	Tofaneli, Freitas, and Pereira (2014)
91.7	0	-	-	VR 043-43	-	Herbaceous	20	0.5	Botelho, Souza, Schreider, Pires, and Terra (2009)
90.3	2000	-	60	-	-	Hardwood	-	-	Somkuwar et al. (2013)
90	250	IBA	24 h	Paulsen 1103	Nutrient solution	Hardwood	45	-	Daskalakis et al (2018)
90	3000	IBA	10	Korinka russkaya		Hardwood			Ismail, Shalamova, and Abramov (2020)
90	500	IBA	20	Ramsey	Sand + peat + sawdust				Mohamed (2017)
90	-	-	-	IAC 313 'Tropical'	Vermiculite	Herbaceous	15	1	Rezende and Pereira (2001)
90	0		5	t140 Ruggeri	Perlite and peat moss				Uzunoglu and Gokbayrak (2018)
88.9	2000	-	60	Riparia of Traviú	Vermiculite	Hardwood	25		Leonel and Rodrigues (1993)
87.5	2000	IBA	3	5BB	Perlite		35		Dogan, Uyak, and Kazankaya (2016)
87.2	1000	IBA	10	VR 043-43	Sand	Herbaceous	15	0.5	Bettoni et al. (2014b)
86.2	2000	IBA	5	Riparia and Rupestris 101-14	Soil + Sand	Hardwood	35		Jesus, Villa, and Chalfun (2018)

84	0	IBA	10	Paulsen 1103	Soil + sawdust + cattle manure	Semi- hardwood	15	0.5	Coradini, Silva, and Korte (2014)
83				IAC 572	Rice husk	Semi- hardwood	14	1	Bordin, Hidalgo, Bürkle, and Roberto (2005)
83	0	IBA	5	Kober 5BB	-	Hardwood	0		Cristoferi, Filiti, and Rossi (1988)
81	8000	IBA		Paulsen 1103	Sawdust	Hardwood	30		Abu-Qaoud (1999)
75	1000		10	SO4	Vermiculite	Herbaceous	5 to 8	2	Nascimento, Dini, Moreira, Sampaio, and Schuch (2019)
73.3	2500	IBA	24 h	Magnolia	Vermiculite	Semi- hardwood	5	1	Biasi and Boszczowski (2005)
73	2000	IBA	5	IAC 572-Jales	Vermiculite	Hardwood	20		Carvalho, Nunes, and Pereira (2014)
69	2000	IBA	5	VR 043-43	Soil		20		Salibe et al. (2010)
68	0	IBA	10	VR 043-43	Sand	Semi- hardwood	20	0.3	Botelho, Maia, Pires, Terra, and Schuck (2005a)
67.6	2000	IBA		140 Ruggeri	Perlite				Bartolini, Fabbri, and Tattini (1988)
64.4				1613C	Sawdust	Hardwood			Soltekin and Altindisli (2017)
57	0	IBA and NAA	15	Riparia of Traviú	Vermiculite	Herbaceous	15		Villa et al. (2003)
43	30	IBA		Ramsey	Phenolic foam	Hardwood	20		Kacar and Isfendiyaroğlu (2019)
41	1000	IBA	5	VR 043-43	Rice husk	Herbaceous	15		Lone et al. (2010)
36.3	0	IBA	15	Bontiful	Vermiculite	Semi- hardwood	10	1	Denega, Biasi, Zanette, Nascimento, and Blaskevics (2009)

Number of articles (n), indole-3-butyric acid (IBA), naphthalene acetic acid (NAA), and indole-3-acetic acid (IAA); leaf of 0.3 (30% of a leaf), 0.5% (50% of a leaf), and 1 (100% of the leaf).

Results and discussion

The results were grouped into four categories to facilitate data discussion. The groups were organized by the effect of the dose and immersion time, cutting type and length, auxin and rootstock, substrate type, and presence or absence of leaves.

Dose and time of immersion

Despite finding that the application of exogenous auxin improves histological characteristics through the formation of calluses and tissues and differentiation of vascular tissue (Mitra & Bose, 1954; Singh, 2018), it may be unnecessary in other circumstances for the grapevine crop. Our results showed that the highest rooting percentages (%) were obtained with the use of exogenous auxin (Figure 1A) at doses of 250, 500, 1500, and 3000 mg L⁻¹, all of which were close to 100%. However, even at a dose of 0 mg L⁻¹, the % values were above 80%. This may imply a recommendation other than the use of exogenous hormones to induce the root cuttings to reduce production costs. Several studies have reported little or no influence of plant growth regulators on the rooting of grapevine cuttings (Goode Jr. & Lane, 1983; Moretti & Borgo, 1985; Thomas & Schiefelbein, 2001; Keeley, Preece, & Taylor, 2003; Thomas & Schiefelbein, 2003; Villa et al., 2003; Keeley, Preece, Taylor, & Dami, 2004; Thomas & Schiefelbein, 2004; Botelho et al., 2005a; Tofaneli et al., 2014; Bartolini et al., 2017). However, our results indicate that there was a slight increase in the percentage of cuttings rooted in the application of exogenous auxins. Thus, its use could be justified when seeking greater rooting efficiency or when there is a limited amount of material available for propagation.

The immersion time of the cuttings in exogenous auxins (Figure 1B) had little influence on rooting. All values were greater than 80%, except for at the immersion time of 15 s. However, studies that aimed at greater efficiency (values above 90%) appeared to use average standard immersion times of 10 s (Ahmed et al., 2017; Chakraborty & Rajkumar, 2018; Nascimento et al., 2019; Ismail et al., 2020) and 20 s (Köse, & Güleriyüz, 2006; Gökbayrak et al., 2010; Mohamed, 2017).

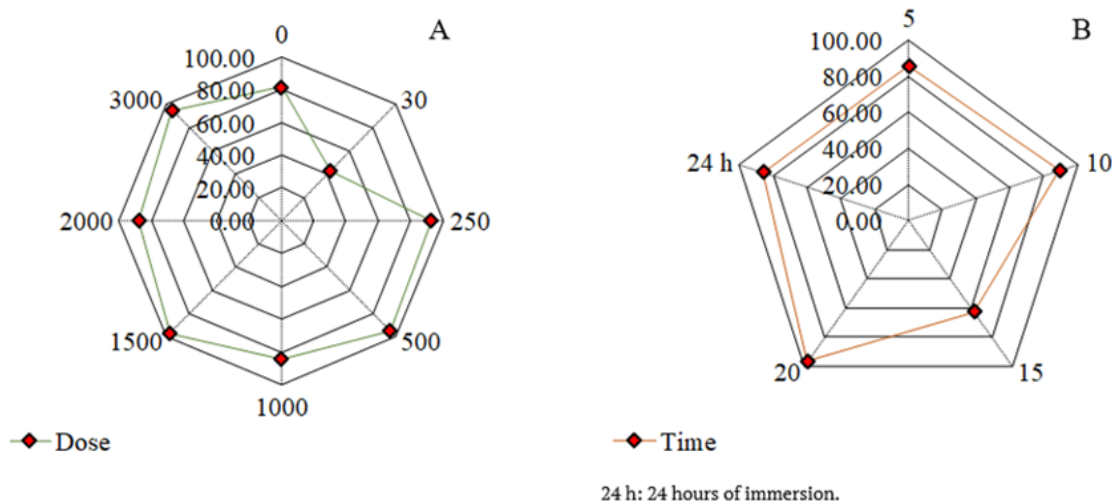


Figure 1. Average highest rooting percentages (%) of grapevine cuttings for dose (mg L⁻¹) (A) and immersion time (seconds) (B) of exogenous auxin.

Cutting type and length

The data for cutting type showed the best results for rooting with hardwood cuttings (Figure 2A). In this cutting type, the average was 91.23%, while the averages for semi-hardwood and herbaceous cuttings were 76.72 and 84.00%, respectively. In general, rooting of hardwood grapevine cuttings is straight-forward (Biasi et al., 1997), and often 100% rooting can be obtained (Da Silva et al., 1986; Roberto et al., 2004c; Köse & Güleriyüz, 2006; Sozim & Ayub, 2006; Amaral et al., 2009; Gökbayrak et al., 2010; Ahmed et al., 2017; Sabir & Sabir, 2018), compared to semi-hardwood (Bordin et al., 2005; Botelho et al., 2005b; Denega et al., 2009) and herbaceous cuttings (Leão, 2003; Villa et al., 2003; Nascimento et al., 2019) with percentageages below 85%. However, with the expansion of cultivation areas, the use of semi-hardwood (Alley, 1980; Egger, Moretti, & Borgo, 1985; Moretti & Borgo, 1985) and herbaceous cuttings (Winkler, Cook, Kliewerer, & Lider, 1974) are viable alternatives to respond to the demand for healthy and high-quality plant material. When there is a need to produce a large number of cuttings from a limited amount of available materials, shorter cuttings and a reduced number of buds can be used.

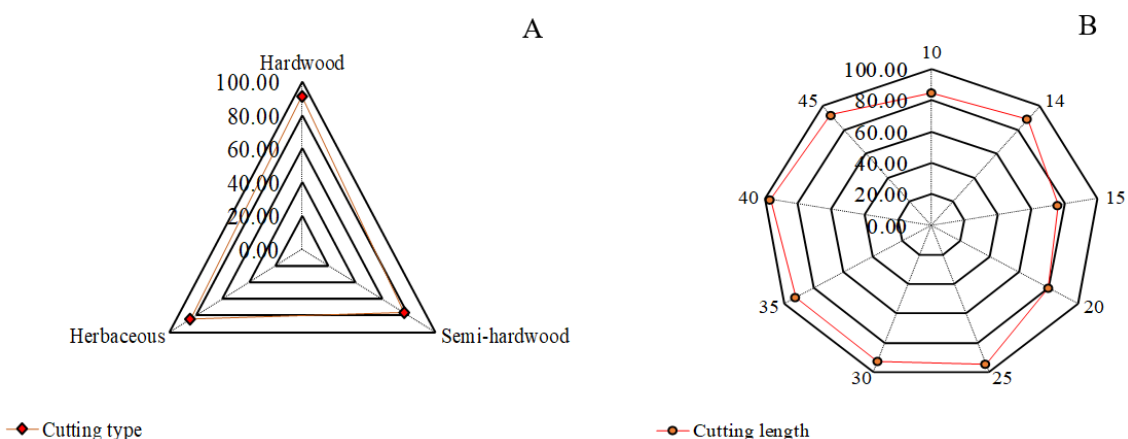


Figure 2. Average highest rooting percentages (%) of grapevine cuttings for type (A) and length (cm) (B).

Figure 2B shows higher values of with increasing cutting length. Cuttings lengths of 25–45 cm showed percentages above 90%. There are two possible reasons for these results. First, it is reasonable to relate the cutting length to the type; Figure 2B shows a direct relationship between the highest percentage and hardwood, which generally has longer cuttings. (Figure 5) (Leão, 2003; Monteguti et al., 2008; Daskalakis, Biniari, Bouza, & Stavrakaki, 2018; Jesus et al., 2018; Daskalakis et al., 2019). There is evidence that the degree of lignification of cuttings has a greater influence on the number of rooted cuttings compared to the length or number of buds per cutting (Leão, 2003). Second, there is likely a greater amount of nutritional reserves present and available for rooting in longer cuttings (Nicoloso, Cassol, & Fortunato, 2001).

Auxin and rootstock

The average value with the use of indole-3-butyric acid (IBA) as an exogenous inducer was 84.3% (Table 3). When combined, indole-3-acetic acid (IAA) and naphthalene acetic acid (NAA) represented only 6% of the selected studies (Table 2). Due to the small number of articles, it is not possible to generate conclusive information about the best auxin for inducing the rooting of grapevine cuttings.

The large number of studies which assessed IBA as a plant growth regulator suggests that this is the main synthetic hormone used for the rooting of grapevine cuttings (Machado et al., 2005). However, factors such as cultivar, time of year, and cutting type, reported 25 years ago (Fachinello, Hoffmann, Nachtigal, Kersten, & Fortes, 1995) still appear to cause variability in the results.

In view of the diverse range of rootstocks available for grapevine propagation, the main four (Table 2 and Figure 3) have a good rooting potential, particularly IAC 766 and Paulsen 1103 with close to 100% success.

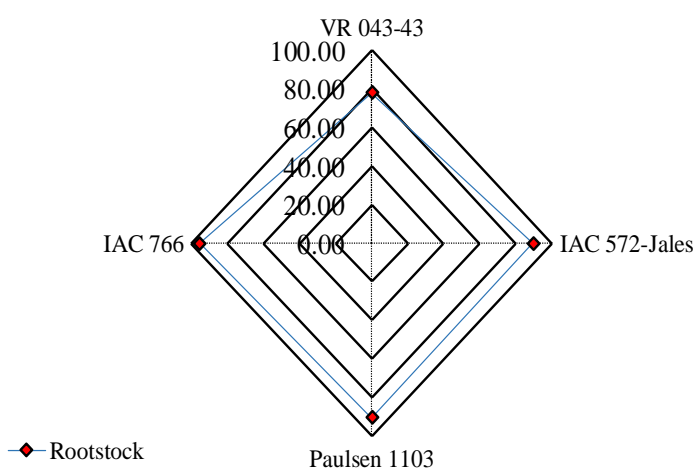


Figure 3. Average highest rooting percentages (%) of grapevine cuttings for rootstock varieties.

Presence or absence of leaves and type of substrate

The presence of leaves proved to be a significant factor in the rooting of cuttings (Figure 4A). Despite the small number of articles in our database that compared rooting with and without leaves (Table 2), it is worth mentioning that the average was above 80% in the presence of leaves (regardless of the measurement unit) (Figure 4A). The presence of leaves in the rooting of herbaceous and semi-hardwood cuttings is essential for obtaining positive results with grapevine (Biasi et al., 1997), showing superior performance to that using hardwood cuttings (Zuffellato-Ribas & Rodrigues, 2001; Botelho et al., 2005b). Genetic materials that are generally challenging to root with hardwood cuttings (Goode Junior, Krewer, Lane, Daniell, & Couvillon, 1982) can provide good results with cuttings in the presence of leaves collected during the plant growth period (Goode Junior & Lane, 1983).

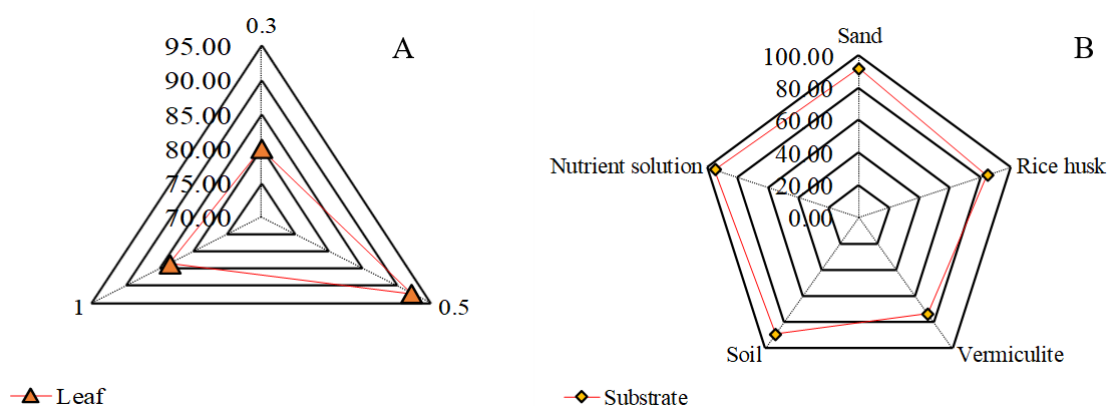


Figure 4. Average highest rooting percentages (%) of grapevine cuttings for 0.3 (30% of a leaf), 0.5 (50% of a leaf), 1 (100% of a leaf) (A) and substrate type (B).

This strong rooting of cuttings is justified by the fact that the leaf is an important auxin source (Hartman, Kester, Davies Junior, & Geneve, 2011), in addition to assisting in the production and transport of carbohydrates from the photosynthetic process to the base of the cutting (Pires & Biasi, 2003). However, the grapevine has large leaves, and a large leaf area is not preferable due to the increased risk of excessive dehydration, which can hinder rooting (Bordin et al., 2005).

The best results were 95, 92, and 89% obtained with nutrient solution, sand, and soil, respectively (Figure 4B). Based on the knowledge of the functionality of the substrate as a means for developing seedlings where structure, aeration, water retention, and absence of pathogens are essential, it is reasonable to conclude that the aforementioned substrates serve these functions well. Furthermore, sand and sandy soils are considered the most widely used substrates (De Albuquerque & Choudhury, 1993). However, despite the high average values, there is evidence of the formation of thick roots with little branching and brittleness when sand is used as a substrate (De Albuquerque & Choudhury, 1993; Hartmann et al., 2011). As a result, it is more advantageous to use soil because of its low cost, increased possibility of forming a healthy root system, and the likelihood of higher rooting percentages, as shown by our research data. However, it is not possible to generalize the use of soil as a substrate for grapevines. Specific recommendations have been made, such as vermiculite for herbaceous and hardwood cuttings (Gonçalves & Minami, 1994) and rice husks for the rooting of herbaceous cuttings of IAC 766 and IAC 572 rootstocks (Roberto et al., 2004a).

Recommendations for the grapevine

Figure 5 shows data on the relationship between the qualitative variable (cutting type) and the quantitative variables (length, rooting percentage, and immersion time). The Microsoft Excel "=AVERAGEIF (QUALITATIVE COLUMN, CRITERION, QUANTITATIVE COLUMN)" function allowed the grouping of this information.

It is possible to make recommendations based on the information presented in Figure 5, such as the fact that it is reasonable to consider applying higher doses of exogenous auxin to hardwood grapevine cuttings, in the absence of leaves at the time of cutting. To achieve a high rooting percentage (close to 100%) with the use of hardwood cuttings of approximately 25 cm (or 3 knots), we recommend using an auxin dose close to or above 1500 mg L⁻¹. However, this implies an increase in production costs with the purchase of exogenous auxin and increased consumption of plant material because of the larger size of the cuttings.

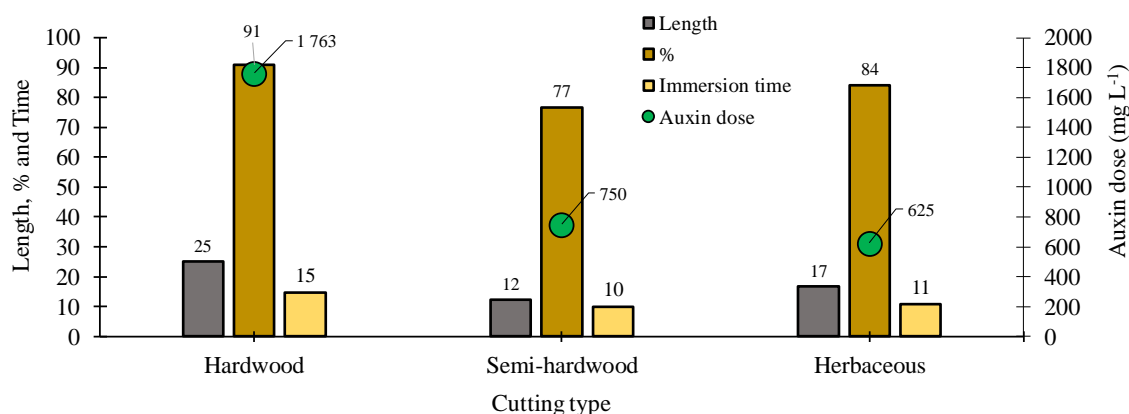


Figure 5. Average length (cm), rooting percentage (%), immersion time (seconds), and auxin dose for different types of grapevine cuttings.

The use of semi-hardwood and herbaceous cuttings for the propagation of grapevine rootstocks can be a viable alternative in situations where the availability of materials is limited, as the smaller size required for these cutting types maximizes the use of materials. Furthermore, the use of cuttings with leaves reduces the need for exogenous auxin application (Figures 4A and 5). Therefore, doses of 500 to 800 mg L⁻¹ are sufficient to meet crop needs and obtain a good rooting percentage (close to 80%), thus reducing the costs for seedling production, and thereby allowing for increased net profit.

Conclusion

The use of hardwood cuttings for the propagation of grapevine rootstocks had the highest rooting percentage of all wood types evaluated, but with a presumed increase in the cost of seedling production due

to the use of synthetic auxins. The presence of leaves in semi-hardwood and herbaceous cuttings increases seedling production efficiency and reduces the cost of purchasing plant growth regulators. For cultivars and/or varieties in which the amount of material available for propagation is limited, the use of semi-hardwood and herbaceous cuttings is recommended.

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