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Differences in rooting ability between wild and cultivated *Vitis vinifera*

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ABSTRACT

Vegetative propagation methods involve using mature plant material to produce new plants. It probably contributed to the key transition for early humans from a nomadic hunter-gatherer existence to settled agricultural communities based on cultivating their plant resources. The domestication of the Eurasian grapevine can be described as a long-term process of selection of suitable genotypes followed by their vegetative propagation. Vegetative propagation through cuttings is one of the earliest known agricultural practices, and historical sources substantiate its widespread use for the cultivation of the Eurasian grapevine. To test whether domestication has had an effect on the grapevine's capacity for rooting and therefore vegetative propagation, 1,061 grapevine cuttings from nine different accessions were studied for 200 days in two different experimental sites in Georgia. Specifically, the vegetative propagation properties of 485 cuttings of the wild vine (*Vitis vinifera* subsp. *sylvestris*) were compared with 576 cuttings of the domesticated vine (*Vitis vinifera* subsp. *sativa*). Subspecies *sativa* showed a higher rooting ability than subspecies *sylvestris*. The domesticated plant also showed a more developed root system characterised by a greater number of first- and second-order roots. Moreover, the domesticated cuttings showed a larger single leaf area, whereas wild cuttings produced more nodes and leaves. The total leaf area for the two subspecies was similar. We propose that the greater rooting ability of the subspecies *sativa* was likely a key character that was selected for during the domestication process, whether more or less consciously. These results provide concrete support for the hypothesis that asexual reproduction plays an important role in the domestication process.

KEYWORDS: cuttings, grapevine domestication, plant propagation, rhizogenesis, rooting ability, *Vitis sativa*, *Vitis sylvestris*

INTRODUCTION

Rhizogenesis refers to the process of root formation in plants, both under natural conditions and in response to certain stimuli in vegetative propagation (Bellini *et al.*, 2014; De Klerk, 2002). It plays a vital role in the ability of plants to reproduce asexually, allowing for the generation of new plants from different parts such as stems, leaves, or roots (Awotedu *et al.*, 2021; Da Costa *et al.*, 2013; Haokip *et al.*, 2023; Singh & Chauhan, 2020). This natural phenomenon is particularly significant in viticulture because different grapevines have been shown to have varying capacities for root formation when propagated vegetatively (Hartmann *et al.*, 2002; Singh & Chauhan, 2020; Waite *et al.*, 2015). Rhizogenesis can occur through two main pathways: adventitious root formation and lateral root formation. In the context of vegetative propagation, adventitious root formation is the primary pathway (Bellini *et al.*, 2014; Li *et al.*, 2009; Pacurar *et al.*, 2014). These roots emerge from cells neighbouring vascular tissues, typically in stems or leaves, and facilitate the plant's ability to grow in new locations (Awotedu *et al.*, 2021; Bellini *et al.*, 2014). The process of rhizogenesis is influenced by a variety of exogenous and endogenous factors, such as temperature, light conditions, hormones (especially auxin), sugars, mineral salts, and other molecules (Druege *et al.*, 2016; Li *et al.*, 2009; Wiesman *et al.*, 2002). Studies have demonstrated that plants with enhanced adventitious root formation show improved propagation success (Druege *et al.*, 2016; Lagos *et al.*, 2022; Li *et al.*, 2009).

The shift from nomadic hunter-gatherer lifestyles to settled agricultural communities that cultivated and produced their own food was most certainly coupled with the development of plant propagation methods. The propagation of perennial plants by vegetative propagation has probably contributed to this change. Techniques of vegetative propagation, pioneered by ancient civilisations such as the Babylonians and Chinese, remain relevant and applicable even in modern agricultural practices (Browse, 1979).

It can be hypothesised that the first training systems in the Near East were designed to facilitate harvesting (Reynolds & Vanden Heuvel, 2009). Initially, the "layering" method (French, *provignage* or *marcottage*) might well have been employed. By burying and directing a shoot growing close to the ground away from the trunk of the vine, it will re-root itself elsewhere. Once the new vine, which is a clone of its mother plant, has sprung up, it can be trained on a support structure. This method was used in western Georgia (Nakashidze, 1896a; Nakashidze, 1896b; Yakovlev, 1896), and is documented throughout Europe today, including Italy, where it has been practised in Roman times and likely much earlier (Geneve, 2001; Seiler, 2024; Thurmond, 2017).

Humans might also have tried to move plants closer to their villages by planting above-ground cuttings, and by chance, found that shoot parts could also regenerate into a complete plant (Zohary *et al.*, 2012), just like layering does. This technique involves using segments of the parent plants (Awotedu *et al.*, 2021; Hartmann *et al.*, 2014) to regenerate new plants.

Vegetative propagation through cuttings is one of the earliest known agricultural practices for perennial plants, as evidenced in the writings of Aristotle (384–322 BC), Theophrastus (371–287 BC), Pliny the Elder (23–79 AD) (Haissig & Davis, 1994). Furthermore, this propagation technique seems to have already been known and described in the book of the prophet Ezekiel (17:22-23) (592–571 BC) in which it is written *"... I will take a twig from the top of the cedar, pluck it from the tips of its branches, and plant it on the high mountain of Israel. It will put forth branches and bear fruit and become a magnificent cedar..."*. Historical evidence of the use of this technique on vines dates back to the 17th century (Jaleta, 2019; Patil *et al.*, 2001; Somkuwar *et al.*, 2011; Wei-June, 1958). Vegetative propagation methods (cuttings, layering, grafting, budding, etc.) (Akinnifesi *et al.*, 2006; Negrul, 1959) enable the bypassing of the prolonged juvenile phase inherent in seed-grown plants, thereby accelerating the onset of fruit production (Asaah *et al.*, 2012; Vivien & Faure, 1996).

Mesopotamian texts dated to around 1800 BC suggest that grapevine shoots were transported for planting, whereas evidence for grafting is thus far lacking at that time (Harris *et al.*, 2002; Lion, 1992; Mudge *et al.*, 2009). Similarly, while agricultural traditions in China go back thousands of years, the first reliable historical records for grafting do not appear until around the first century BC (Mudge *et al.*, 2009). Based on the available historical and archaeological evidence, cuttings and layering remained the primary propagation methods until the middle of the nineteenth century, before the *Phylloxera* invasion and transferring the viticulture from self-rooted plants to grafted communities (Mudge *et al.*, 2009). It has been argued that farmers in the Near East, over 6,000 years ago, were already using cuttings to propagate figs, grapevines, pomegranates, and olives, ensuring crop consistency and preserving desirable traits (Mudge *et al.*, 2009; Shahal *et al.*, 2022; Zohary & Spiegel-Roy, 1975). According to Zohary and Spiegel-Roy (1975), the first domesticated fruit crops would have been easily capable of vegetative propagation. Other fruit crops, like apples, peaches, cherries, and others, were domesticated only after thousands of years, when the people had mastered grafting techniques (Mudge *et al.*, 2009).

Grapevine, particularly benefited from the cutting and layering methods, as it enabled the preservation of fruit quality and traits to be transmitted between generations (Haokip *et al.*, 2023). Beyond food production, cuttings were also used for ornamental and flowering plants, enabling the spread of preferred varieties to different regions rapidly (Meher *et al.*, 2021).

The Eurasian grapevine is more than a fruit crop. As expressed in its principal product, wine, it has been the basis for "wine cultures" in which the beverage dominated socio-economic structures, religious beliefs, artistic creations, and pharmacopoeias (McGovern, 2024).

Among these, *Vitis vinifera* stands out as the only species extensively cultivated in the global wine industry and is indigenous to western Eurasia. This species includes two recognised subspecies: the wild form, *V. vinifera* subsp. *sylvestris* (Gmelin) Hegi. And the domesticated form, *V. vinifera* subsp. *sativa* DC. The *sylvestris* subspecies is regarded as the ancestor of the domesticated *sativa*, and the two subspecies differ phenotypically in flower, seed, and leaf morphology, as well as in berry and bunch size (Maghradze *et al.*, 2021; This *et al.*, 2006).

The earliest biomolecular archaeological and archaeobotanical evidence of wine production has been found in the South Caucasus region, where early humans are hypothesised to have also begun selecting wild grapevines for desirable traits such as fruit yield and possibly ease of propagation (McGovern, 2017). It has been proposed that vegetative propagation has been a key factor in the domestication and spread of grapevines, enabling the development of diverse cultivars over time (McGovern, 2017; McGovern, 2024; Myles *et al.*, 2011). Thus, it has been further hypothesised that the selective propagation of strong-rooting vines laid the groundwork for modern grapevine propagation techniques, underscoring the importance of vegetative propagation in sustaining grape production from the domestication period up to the present (McGovern, 2013). However, while *Vitis vinifera* subsp. *sylvestris* is widely acknowledged as the wild ancestor of domesticated grapevines; there is little direct research on its natural rooting ability. To the best of our knowledge, no experimental studies specifically addressing the rooting ability of *V. vinifera* subsp. *sylvestris* have been documented in the existing literature.

Considering that the separation between the two subspecies probably occurred during the domestication process, we aim to test the rooting ability of *V. vinifera* subsp. *sylvestris* in comparison to *V. vinifera* subsp. *sativa*. We hypothesise that rooting ability was selected during the domestication process, thus providing evidence that vegetative propagation was commonly used during the early Neolithic period.

MATERIALS AND METHODS

1. Location, plant material and experimental design

The experiments were conducted during 2024 at the experimental vineyards of Jighaura and Okami, located in the Inner Kartli region of Georgia in the South Caucasus (Figure 1). Okami, an experimental farm of the Caucasus International University, is in a temperate climate zone, characterised by moderately cold winters and hot summers. The average annual temperature is 11.4 °C. The total active temperature range is between 3,450 °C and 3,600 °C, with annual precipitation averaging 500 mm. The region features meadow brown, carbonate, and alluvial soils. The soil texture primarily consists of heavy and medium loams and light clays, with the clay content ranging from 45 % to 85 %. Carbonates are present in small amounts, varying between 2.8 % and 6.6 %. The soils are predominantly alkaline, with a pH ranging from 8.2 to 8.7 (National Wine Agency, 2022). Jighaura—an experimental farm of the Scientific Research Centre of Agriculture—is in a temperate climate zone, characterised by cold winters and hot summers. The average temperature is 10.0 °C, and the annual total of active temperatures ranges from 3,440 °C to 3,670 °C. The average annual precipitation is between 540 mm and 590 mm. The soil is characterised by loose, sometimes pebbly, granular-concrete brown soils, which have good physical properties and moisture retention. The soil pH ranges from 7.8 to 8.1, and the organic matter content in the soil is between 1.4 % and 1.6 % (Kikilashvili, 2022).

A total of 1,061 cuttings representing nine accessions were studied: they included four varieties of *Vitis vinifera* subsp. *sativa* (576 cuttings) and five genotypes of *Vitis vinifera* subsp. *sylvestris* (485 cuttings) (Table 1). The dormant woody canes for propagation were collected in Jighaura's ampelographic collection (the FAO code of the collection is GEO038) in the late winter-early spring of 2024 and were stored in a refrigerator at 5 to 7 °C until starting an experiment. In the following days (April, 17 and 18, 2024), cuttings with 3–4 nodes and about 25–30 cm in length were

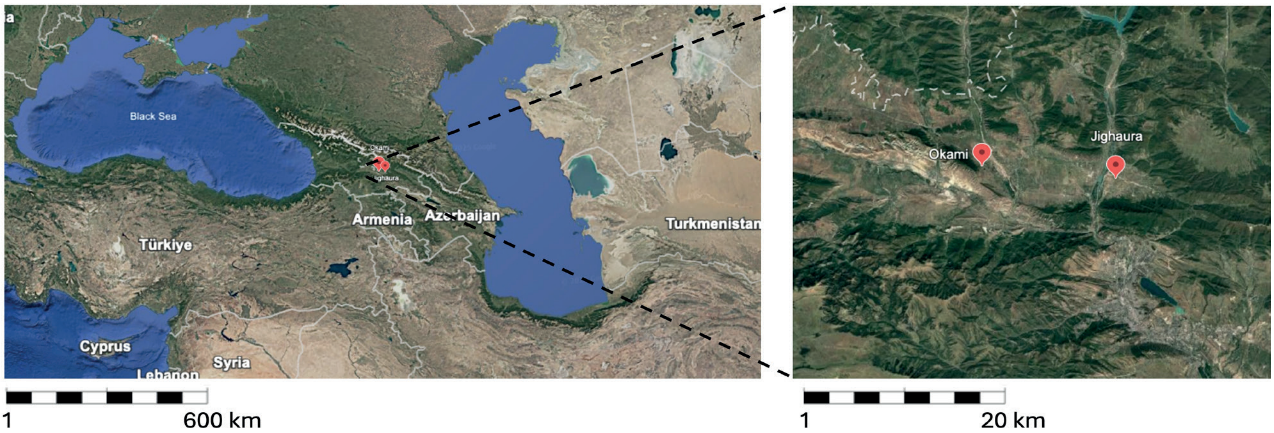

FIGURE 1. Satellite images of the Caucasus region (left) and the Georgian region (right) showing the location of the sites under study (Google Earth).

prepared for the experiment. The apical portion of the cutting was paraffin-embedded to avoid dehydration, and the basal portion was soaked for 24 hours in a 0.1 % water solution of 4-(indol-3-yl) butyric acid (Kornevin® SP, Agrozintez, Moscow), a synthetic auxinosimilar rooting stimulant. Before the cuttings were planted, the soil was tilled with a spade to a depth of 20–30 cm. A drip irrigation system was installed with mulching under black plastic coverings. Cuttings were planted on April, 25 at the Okami experimental site and on April, 20 at the Jighaura experimental site.

Two replications comprised of subgroups of cuttings, were conducted for each location (Okami and Jighaura). More information on the populations of each replicate is shown in Table S1 in the Supplementary data. Six copper and sulfur treatments were made during the growing season to control downy mildew and powdery mildew.

2. Data collection

Before planting the cuttings, the diameter of each was measured in the middle portion with a calliper. Then, for each experimental site (Okami and Jighaura) and for each individual accession, the total weight of the cuttings was recorded in the AMW-2000 digital scale (D. 0.1 g). Dividing the total weight by the number of cuttings, the average weight of the cuttings was determined. Sprouting and the growth parameters of the cuttings were recorded during the vegetative growing season, as measures of propagation success. Before phylloptosis (*i.e.*, the fall of the leaves, mid-October), the area of each leaf was measured using "Easy Leaf Area" software, as described by and already used in grapevine descriptions by Dinu *et al.* (2021). At the end of the vegetative growth period, each rooted cutting was explanted, and the root system analysed: the number of first-order and second-order roots were counted, and, using a ruler, the average length of first-order roots was measured. The propagation success was calculated for each genotype by evaluating the budburst of the cuttings and the survival of the newly developed shoots during the 200-day observation period. Finally, the number of buds, and thus nodes/leaves, for each shoot was determined, and the total leaf area was estimated by multiplying the average leaf area value by the number of nodes.

3. Statistical analysis

Data on the rooting ability of the cuttings were analysed using Student's *t*-test for each growth time, while a repeated-analysis ANOVA was used to evaluate the interaction between subspecies and time (ns = not significant, while *, **, ***, represent significant differences at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively). When differences among subspecies were significant, the means were separated using the *post hoc* Tukey's Honest Significant Difference (HSD). The data were first standardised by expressing them as a function of days of growth from the planting of the cuttings (T0) with constant development intervals of 25 days (T25, T50, T75, T100, T125, T150, T175, and T200). Other data (diameter and weight of cuttings, number of first- and second-order roots, the average length of first-order roots, leaf area, bud load, and total leaf area) were compared by Student's *t*-test; where assumptions of normality (using the Shapiro–Wilk test) or homoscedasticity (using Levene's test) were not met, the analysis was performed by Welch's *t*-test (in which the data are normally distributed but have unequal variances) or Wilcoxon's test (non-normal data). All the statistical analyses and graphs were done using R software (R Core Team, 2022; version 4.4.1).

TABLE 1. List of accessions of *Vitis vinifera* subsp. *sativa* and *Vitis vinifera* subsp. *sylvestris*, documenting flower sex and the number of cuttings used in the rooting trials. A comparable number of plantings was done at the Okami and Jighaura experimental sites.

Accession	Sex of flowers	N° of cuttings planted in	
		Okami	Jighaura
Vitis vinifera* subsp. *sativa			
Cabernet franc	Hermaphrodite	75	77
Ojaleshi	Hermaphrodite	78	75
Pinot noir	Hermaphrodite	78	77
Saperavi	Hermaphrodite	58	58
Vitis vinifera* subsp. *sylvestris			
Bagichala 07	Male	34	33
Chachkhriala 01	Female	67	65
Nakhiduri 15	Female	62	61
Tedotsminda 03	Male	26	27
Tedotsminda 22	Male	54	56

RESULTS

The diameter and weight of *Vitis vinifera* subsp. *sativa* and *sylvestris* (Table 2) cuttings used in the study were determined before planting. Statistical analysis showed no statistically significant difference between the specimens belonging to subspecies *sativa* and *sylvestris*.

The rooting ability of *V. Vinifera* subsp. *sylvestris* and *V. Vinifera* subsp. *sativa* cuttings were monitored over a period of 200 days by measuring the percentage of live cuttings as a function of time (Figure 2). In the first 50 days (T0–T50), no statistically significant differences were noted between the two species, with both subspecies *sativa* and *sylvestris* showing similar trends in their cuttings' survival. However, from T75 onwards, the percentage of live cuttings started to differ significantly, with consistently higher values for *V. vinifera* subsp. *sativa* than for *V. vinifera* subsp. *sylvestris*. At the end of the observation period (T200), the proportion of live cuttings of cultivated grapes was 49.4 %, compared to 33.2 % of *V. vinifera sylvestris*. This proportion was consistent at both experimental sites, Okami and Jighaura, as shown in Figure S3 in the Supplementary data.

Analysis of the root system at the end of the vegetative growth period showed important differences among *V. vinifera* subsp. *sativa* and *V. vinifera* subsp. *sylvestris* (Figure 3A). Whereas *sylvestris* cuttings had more first-order roots at the lower end of the scale (0–5), *sativa* cuttings peaked at a higher number (5–10). At even higher numbers (> 10), the probability density distributions of the two subspecies were similar. Statistical analysis confirmed a significant difference between the two subspecies ($p = 0.038$, *). In contrast to the significant differences observed in root number, the average length of first-order roots did not show statistically significant differences between *V. vinifera* subsp. *sativa* and *V. vinifera* subsp. *sylvestris*, as indicated by the overlapping probability density distributions (Figure 4).

The analysis of second-order roots (Figure 3B) showed an even more significant difference between subsp. *sativa* and *sylvestris* ($p = 4.03 \times 10^{-07}$, ***) than that for the first-order roots. *Sativa* cuttings exhibited a broader distribution of higher root numbers, while *sylvestris* showed a more concentrated density at the lower end of the range. The median number of second-order roots was substantially higher in *sativa* compared to *sylvestris*, confirming the greater capacity of *sativa* cuttings to develop a more extensive root system.

TABLE 2. Mean diameter and weight of cuttings (expressed as mean ± standard deviation) for *Vitis vinifera* subsp. *sativa* and *Vitis vinifera* subsp. *sylvestris* before the rooting experiment. The statistical significance value for diameter was assessed by Welch's test, while Student's *t*-test was used for weight.

Cuttings	*Vitis sativa*	*Vitis sylvestris*	Level of significance	Type of statistical test
Diameter, mm	5.98 ± 1.07	6.00 ± 1.22	ns	Welch's test
Weight, g	10.23 ± 1.23	10.13 ± 1.55	ns	Student's *t*-test

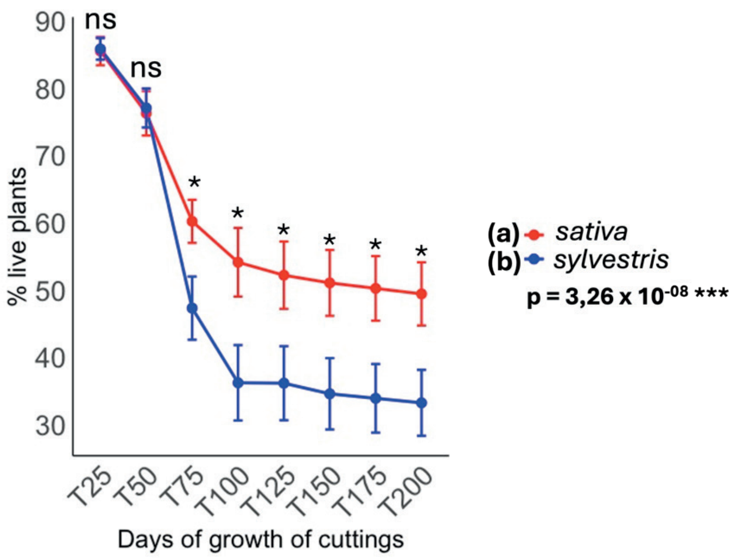

FIGURE 2. Percentage trend of live cuttings of *Vitis vinifera* subsp. *sativa* (in red) and subsp. *sylvestris* (in blue) as a function of days (T25, T50, T75, etc.). Differences between the two subspecies were evaluated by Student's *t*-test at each sampling time, while a repeated-measures ANOVA (Repeated ANOVA) was performed to determine the interaction between subspecies and time. Where the ANOVA showed significant effects, a Tukey's HSD test was used for mean separation. Error bars indicate the standard deviation. Asterisks specify the level of significance (*** = $p \leq 0.001$; ** = $p \leq 0.01$; * = $p \leq 0.05$), and different letters indicate statistically different averages.

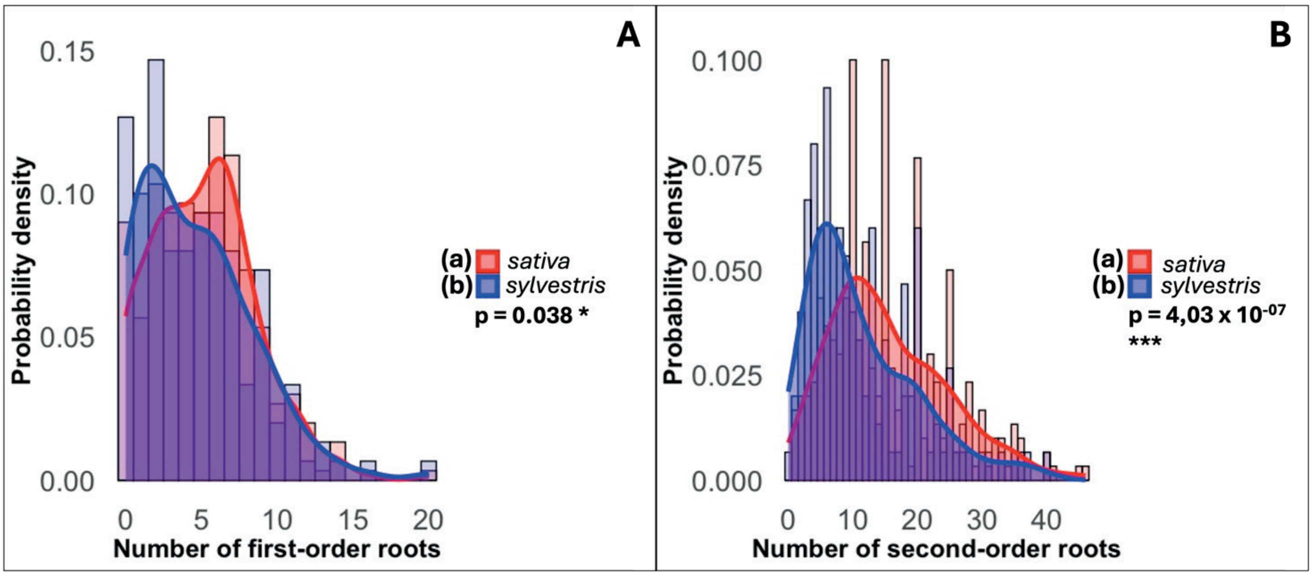

FIGURE 3. Frequency distribution of the number of first-order (A) and second-order (B) roots for *Vitis vinifera* subsp. *sativa* (in red) and subsp. *sylvestris* (in blue). The horizontal axis shows the number of roots of each order, and the vertical axis shows the probability density.

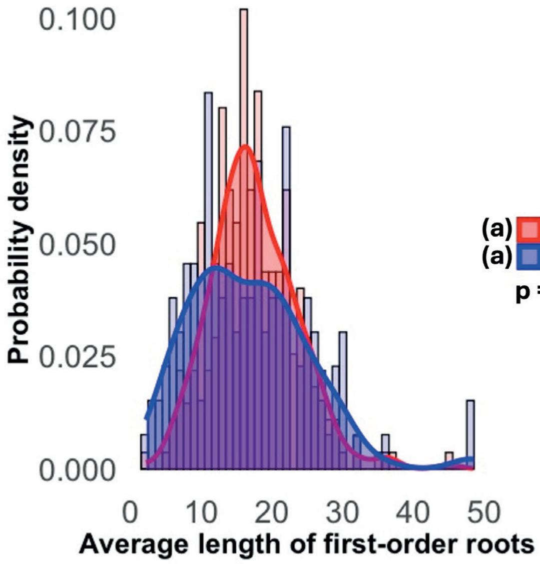

FIGURE 4. Frequency distribution of the average length of first-order roots for *Vitis vinifera* subsp. *sativa* (in red) and subsp. *sylvestris* (in blue). The horizontal axis shows the number of roots of each order, and the vertical axis shows the probability of density.

Observations conducted at the end of the vegetative growth period of the plant (Table 3) show that cuttings of *V. vinifera* subsp. *sativa* have significantly higher median values of the average leaf area than those of *V. vinifera* subsp. *sylvestris*. In other words, the cultivated grapevines generally have larger leaves than the wild ones. In contrast, the median number of buds per shoot (bud load) is higher in subspecies *sylvestris*, indicating that the latter produced more leaves. Consequently, although wild grapes have a smaller leaf size, the greater total number of leaves of subspecies *sylvestris* results in the total leaf area of the two subspecies being comparable, with no statistically significant difference.

DISCUSSION

Vegetative propagation by cuttings is one of the most widely used practices in the horticultural industry (Patil *et al.*, 2001) and was historically also in viticulture (Somkuwar *et al.*, 2011). According to Levadoux (1956) and Grassi and De Lorenzis (2021), the cultivated grapevine (*Vitis vinifera* subsp. *sativa*) originated from the domestication of wild populations of *Vitis vinifera* subsp. *sylvestris*. The grapevine was likely domesticated to produce wine sometime during the early Neolithic period (*ca.* 6000–5800 BC) when humans had begun to settle

TABLE 3. Mean values (± mean simple deviation from median) of four parameters (average leaf area, bud load, total leaf area, and plant weight) in *Vitis vinifera* subsp. *sativa* and subsp. *sylvestris* at the end of the vegetative season. The level of significance was calculated by using the Wilcox test. Different letters indicate statistically different averages.

Parameter	*Vitis sativa*	*Vitis sylvestris*	Level of significance
Average leaf area, cm^2	47.9^a ± 15.4	32.9^b ± 12.7	***
Bud load per vine, number	15.0^b ± 7.53	23.5^a ± 13.2	***
Total leaf area per vine, cm^2	703 ± 463	752 ± 585	ns
Plant weight, g	30.1 ± 17.81	30.31 ± 20.21	ns

down and could care for the plants, and when pottery had been invented and provided better means to produce and preserve the beverage (McGovern, 2017). However, the dating of the initial domestication of the grapevine is still the subject of much debate. In this context, Deckers *et al.* (2024) suggest that the earliest indications for grape cultivation appear in the fourth millennium BC in Southwest Asia. This is a region that exhibits the characteristics of a primary domestication centre, including a long-standing interest in viticultural development, a relatively high population density, well-established settlements, and a strategic location at the intersection of trade routes and cultural interaction (McGovern *et al.*, 2017). Modern genetic studies pointed Near East and the South Caucasus as the main centres for the wine grape domestication (Dong *et al.*, 2023), provisionally 11,000 years ago. The domestication process involved selecting genotypes with larger berries and bunches through sexual reproduction, but to preserve the varieties, it most likely relied on the development of vegetative propagation techniques (Maghradze *et al.*, 2021; Zohary *et al.*, 2012). However, during cultivation and domestication, other traits were also probably selected for (Bacilieri *et al.*, 2013), such as ease and effectiveness of vegetative propagation. The discovery of this propagation method was likely of great significance, allowing the first grape growers to stabilise the plantation of heterozygote grapevine for better agronomical practice. Our study investigated potential differences in the process of rhizogenesis and thus the rooting ability of cuttings obtained from different accessions of *Vitis vinifera* subsp. *sylvestris* compared with subspecies *sativa*.

By observing specific characteristics of live plants over time, we were able to identify statistically significant differences between the rooting ability of wild and cultivated vine cuttings. Although both subspecies showed a high bud break percentage in the first 50 days, from day 75 onward, the survival rate declined more sharply in *V. vinifera* subsp. *sylvestris* (–30 %) compared to *sativa* (–16 %). To avoid possible impacts of the physical dimensions of the cutting size on rooting viability and plant survival, similarly sized stem portions were selected from both subspecies, ensuring no significant differences in the cuttings at the start of their growth (Table 2). The marked decrease in living plants observed in our study could then be due to the demand for resources, such as carbohydrates, necessary to support epigeal growth before hypogeal apparatus creation. In fact, the rooting process is carbohydrate-intensive (Tombesi *et al.*, 2015). Additionally, the buds might have begun to develop and send out new shoots before the root system of a cutting was formed or was insufficiently developed, resulting in the plant being unable to absorb adequate water and nutrients. These limitations might in turn have led to the progressive desiccation and death of the shoots. This imbalance between shoot and root development may explain the higher mortality observed in *V. vinifera* subsp. *sylvestris*. The percentage of live plants in subsequent days of growth was consistently higher in the group of subspecies *sativa* than in subspecies *sylvestris*, thus demonstrating that the rhizogenesis process of the cuttings from cultivated plants was more effective than the wild plants.

The results obtained in our study clearly show that *V. vinifera* subsp. *sativa* developed a more branched root system than *V. vinifera* subsp. *sylvestris*, both in terms of the number of first-order and, to an even greater extent, second-order roots. Functionally, the ability to develop a higher number of roots and generate a larger branching network can be traced to the geometric, topological, and morphological traits of the roots. Characteristics such as branching density and lateral root expansion have been widely correlated with the plant's ability to acquire resources and maintain adequate physiological functions even under abiotic or biotic stress conditions (Bardgett *et al.*, 2014).

In our study, the growth of the two subspecies differed mainly in the hypogeal apparatus, while the epigeal growth ensured a similar total leaf area despite the morphological differences among the plants, *i.e.*, the wild grapevines had many small leaves and the *sativa* subspecies had a few large leaves. Therefore, it can be hypothesised that the domestication process impacted both the morphology of the epigeal part of the plant and the physiology of the hypogeal apparatus. There is usually a common suite of traits designated as the "domestication syndrome" that distinguishes most seed and fruit crops from their progenitors (Hammar, 1984).

Reconstructing ancient agricultural techniques is inherently challenging due to the scarcity of direct archaeological evidence, particularly regarding propagation methods. Nevertheless, the functional differences observed between wild (*V. vinifera* subsp. *sylvestris*) and cultivated (*V. vinifera* subsp. *sativa*) grapevines offer crucial insights into the probable practices employed during the earliest stages of domestication. Our results clearly indicate that subspecies *sativa* exhibits a superior rooting ability compared to subspecies *sylvestris*, a feature that likely facilitated its selection and widespread adoption at the dawn of viticulture. These findings support the hypothesis that vegetative propagation through cuttings was not only feasible but might well have been actively exploited during the grapevine domestication period, serving as a strategic advantage in maintaining and diffusing desirable traits. In addition, the pronounced divergence in rooting capacity supports the view that domestication favoured genotypes with enhanced rhizogenesis, improving both the survival and performance of propagated cuttings. Thus, the greater propensity of subspecies *sativa* to develop more robust and branched root systems could be seen as a direct evolutionary outcome of human-driven selection, and it can be considered a newly described trait of the grapevine domestication syndrome. Further studies, involving different multidisciplinary approaches, could further clarify the propagation techniques in ancient times.

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